STUDIES ON THE ECOLOGY OF STAPHYLOCOCCUS AUREUS

AND THE EPIDEMIOLOGY OF STAPHYLOCOCCAL INFECTIONS

by

James Cameron Gould, B.Sc. M.B.Ch.B.

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INTRODUCTION

The differentiation of strains of Staphylococcus aureus isolated from infections and elsewhere in the environment has been difficult in the past because of the lack of suitable laboratory techniques for testing easily recognised and stable strain characters. This has made the epidemiological study of staphylococcal infections unsatisfactory and the resulting lack of precise knowledge of the sources and routes and modes of spread has interfered with the development of successful preventive methods.

More recently the use of techniques such as the coagulase test and bacteriophage and serological typing tests have been more successful in defining strains and are being used more and more to study the epidemiology of staphylococcal infections.

In recent years staphylococcal infections have received more attention than in the past, due to an apparent increase in their incidence, especially in hospital, and also because of the ability of Staphylococcus aureus to adapt itself to anti-bacterial agents which have been introduced for its control. There is need therefore for a detailed investigation of the sources, routes and modes of spread of staphylococcal infection and /

and the part played by antibiotics in their epidemiology.

Staphylococcal Infections

Staphylococcal infections are common and occur throughout the population at home, at work and in hospital. The great majority of these infections are superficial in character and include a wide range of clinical appearances. For long they have been looked upon as relatively minor ailments which, in most cases resolve spontaneously so that serious consequences are few, and death rare. Infections in which the organism is more deeply situated or more widespread throughout the tissues are relatively rare but they are potentially serious.

On the basis of their cause, Staphylococcus aureus, all of these infections may be regarded as a single bacteriological or clinical entity, all being examples of a contagious disease similar to diphtheria, dysentery or streptococcal infections. In common with other important bacterial diseases such as those due to the intestinal pathogens, human carriage of the organism is common, but overt disease relatively rare. Thus Staph. aureus must be regarded as a commensal organism with pathogenic properties and infection must be due to an alteration in equilibrium existing between parasite and host.

There can be little doubt that the staphylococcus is an important organism causing many infections among the general community. Localised staphylococcal lesions such as pustules, furunculosis, carbuncles, styes and many others are a common experience of all practitioners. A very small number of more

serious /

serious and generalised staphylococcal infections are seen. However the number of investigations of these conditions in general practice has been very few and it is difficult to estimate the actual incidence of staphylococcal infection among the general population and the part these infections play in human discomfort, disability and loss of earning power. The reports that are available indicate the importance of outbreaks of staphylococcal infection in families (Harrison,1948) which have all the features of localised epidemics of a contagious disease, and some attention has been paid to the frequency of infection occurring in wounds in factories (Brit. med. J. 1955). More recently outbreaks of staphylococcal skin infection such as impetigo have been described in children where conditions were suitable for spread as in school (Parker, Tomlinson & Williams,1955).

However, it has been in hospital that most attention has been given to staphylococcal infections and it is possible that we may be recording things that previously escaped attention. Twenty-five years ago the major disease producing micro-organisms in hospital were Streptococcus pyogenes and Staphylococcus aureus. Now that infection with the haemolytic streptococcus is restricted to relatively rare, isolated cases or small outbreaks, Staph. aureus has become the commonest and most important single organism causing hospital infections.

In hospital the most important groups of infections are those /

those that occur post-operatively in wounds, infants and mothers in maternity units, in burns, and among members of hospital staff. Staphylococcus aureus is found as the single infecting organism in the majority of all of these infections and occurs in most of the remainder. All are regarded as cross-infection. Most often they are clinically mild but occasionally have serious results which is more often the case in thoracic and plastic surgery and in maternity units (Timbury, Wilson, Hutchison and Govan, 1958).

It is held by many that staphylococcal sepsis of a minor nature is commoner than it used to be in hospital. However staphylococcal infection is not new (Smith, 1935, Lancet, 1935, 1936a), nor is the necessity for closing wards to limit the spread of infection (Lancet, 1936b). Hart (1937) found that 90 per cent of sporadic operation room infection was due to Staph. aureus and Miles (1941) found that as many as 90 per cent of wounds in a ward may be contaminated with the organism. Staphylococcal infections assuming epidemic proportions in both maternity and surgical units have often been described (Knott and Blaikely, 1944) as have outbreaks of breast abscess and staphylococcal infection of the new-born (Hobbs, 1944). However, Howe (1956) reported that surgical wound sepsis increased from 2-7 per cent between 1949 and 1953 in certain Boston hospitals, and many other reports have indicated wound infection rates in excess of 10 per cent in hospitals /

hospitals in this country and abroad. (Knott and Blaikley,1944 ; Hobbs,1944, Delafield, Parry and Gillespie,1954 ; Jeffrey and Sklaroff,1958). It becomes increasingly clear however that the recorded incidence of hospital infection due to the staphylococcus depends very much on the criteria used to define infection, and the care taken in looking for minor sepsis, so that the making of comparisons is difficult.

In hospital, staphylococcal infection has always been regarded as serious in its potentialities, and when generalised infection ensued in the period before chemotherapeutic agents became available, the mortality approached 75 per cent (Lancet, 1939). The introduction of antibiotics for therapeutic purposes was expected to lead to the control of staphylococcal infection, for penicillin was the first antibacterial agent with a strong anti-staphylococcal effect in the patient. It is probably true that for a time staphylococcal infections in patients were controlled by penicillin and the incidence in hospital temporarily reduced (Verney). Within the past few years however, it would appear that the incidence of severe staphylococcal infection has increased (Bondi, Pfaff, Free &. Swerlick,1954) due to antibiotic resistant strains of Staph. aureus and many of these cases become concentrated in hospital. Particularly important are cases of staphylococcal pneumonia which may follow primary viral or bacterial pneumonia (Maccabe,1959). It is relatively common in young children and also in adults admitted to hospital during influenzal /

influenzal epidemics, or those with exacerbations of chronic respiratory tract disease, and it may also complicate surgical operations as a post-operative hazard. Staphylococcal enteritis, endocarditis and bacteraemia secondary to primary foci have all increased (Lancet,1952) and this may be specifically related to the therapeutic and prophylactic use of antibiotics.

Carriers of Staphylococcus aureus

Staph. aureus can be isolated from many sites in the environment such as the dust and fomites, clothes, bedding and drapes. The numbers are usually small and seem to indicate spread from some more heavily populated source. Recent investigations have made it increasingly obvious that man himself is the chief source of these organisms dispersing them either as a healthy carrier or as a person suffering from staphylococcal infection. To a certain extent this was appreciated by bacteriologists many years ago and Ogston (1882) remarks that 'it is his conviction that the common micrococci that exist on us, sometimes enter (the body) under conditions of lessened vitality .....and are one and the same with the virulent cocci that cause inflammation'. There is no need, he says, 'to call into account solids, fluids and gases around us in our search for sources of infection when we possess in our own frames so abundant a supply'.

Numerous investigators have examined the surface of the human /

human body for pathogenic staphylococci and in a certain number of persons large numbers of these organisms can be isolated from particular sites such as the nose (Thomson and Hewlett,1895; Bloomfield,1921.) and these persons have been regarded as carriers (Miles,1941; Moss, Squire and Topley,1948 and Lepper, Jackson and Dowling,1955).

McFarlan (1938) found about one-third of the general adult population were nasal carriers but thought that the great majority of healthy individuals are carriers from time to time. Since then the nasal carrier rate has been determined by many investigators and, based on single examinations averages 85 per cent for infants (Miles, 1941; McFarlan,1942; Martyn,1949; Thomas and Cunliffe,1949; Doranyi,1955; Duncan and Walker 1949; Turpin, Pillet and Pillet,1953; Ludlam,1953; Hurst 1957; Cook, Parrish,Shooter,1958); 51 per cent for children (Hallman, 1937; McFarlan,1938; Gillespie, Devenish and Cowan,1939; Miles, Williams and Clayton-Cooper,1944; Rountree, Barbour and Thomson,1951); 50 per cent for adults (Hallman,1937; McFarlan,1938; Miles et al,1944; Williams, 1946 ; Thomas and Cunliffe,1949; Doranyi,1955; Rountree and Thomson,1949, Elwood,1951; Vlasack,1951; Elwood,1951; Rountree, Freeman and Barbour,1954); and /

and 65 per cent for hospital staff (Hart,1937; Devenish and Miles, 1939; Alison and Hobbs,1947; Barber and Rozwadowska-Dowszenko, 1948; Rountree and Thomson,1949; Rountree, Barbour and Thomson, 1951 Martin and Whitehead,1949)

If the examinations are frequent enough and coer a sufficiently long time nearly all persons will yield Staph. aureus from the nares once or more butthe number who yield it on successive samplings is considerably less (Rountree and Barbour,1951, Williams,1946.) and repeated examination of the same individuals has shown that the presence of the organism is sporadic in some and constant in others.(Williams,1946) . Others are persistently free(Hutcheson, Green and Grimson,1957; Rountree and Barbour,1951) their nares being colonised by Coagulase negative Staphylococci (Lepper, Jackson and Dowling, 1955). Therefore the majority of swabs positive on merely a single occasion indicate temporary contamination, or at most temporary colonisation of the nares. The figures for nasal carriage rates given above are therefore too high as they will include a proportion of temporarily contaminated persons, and it is proper to confine the term carrier to those who persistently harbour the organism.

Staph. aureus can be frequently recovered from skin and other sites than the anterior nares, but the numbers isolated are usually relatively small and their presence depends on colonisation of the nose (Gillespie, Devenish and Cowan,1939, Martin and Whitehead, 1949) since only 1 -2 per cent of persons with Staph. aureus on their /

their skin surface did not carry the organism in the anterior nares (Devenish,1939). Gotz (1951) used suction to sample organisms from the pores of the skin of healthy individuals, but of 196 strains of cocci isolated only 2 per cent were Staph. aureus so the number of skin carriers must indeed be small. Williams (1946) carried out a more thorough and prolonged study of nasal and skin carriage using phage typing techniques to identify the strains, and confirmed that in most persons the same staphylococci are present on the hands as in the nares.

Other sites may be colonised in addition to the nose. Hurst (1957a) showed that in nasal carriers a significant proportion had sufficient numbers in the throat to render them capable of acting as donors, and Hare and Ridley (1958) found the perineum to be a source in many persons with and without concomitant colonisation of the nose.

Staph. aureus may be isolated from many specimens of faeces using selective media (Chapman,1945; Ludlam,1949; Maitland and Martyn,1948; McFarland,1949, Fairbrother and Southall,1950) but the numbers in most specimens of faeces must be small. Greendyke (1958) found the same strain of Staph. aureus in the nose and faeces of 40 per cent of nasal carriers so that it is likely that the majority of nasal carriers swallow the organism and in some this may lead to colonisation of the perineum (Hare and Thomas,1956).

Most investigators agree that the nasal carrier rate is higher in persons in hospital than among those in the general community, and that the carrier rate increases with the period spent living inside /

inside hospital (Cunliffe,1949; Laurell and Wallmark,1953 a,b,; Brodie, Kerr and Somerville,1956 ; Thomson and Gillespie,1958 ; Rountree and Barbour,1951; Vogelsang,1953; Rountree and Barbour,1951; Knight, White, Foster and Wenzl,1956, Goslings and Buihli,1958) Clark(1957) found that a person with a nose containing no Staph. aureus was more liable to acquire Staph. aureus than one already containing the organism. Knight and White (1958) on the other hand found non-carriers in hospital persistently resistant to colonisation. Torrey and Reese (1945) found that there was rapid colonisation of the newborn so that 90 per cent yielded profuse growth from the anterior nares on the 8th day after birth. This has been frequently confirmed (Kneeland,1930; Levesque and Pecker,1952, Loh and Abiog 1957). These findings probably reflect the increased chances of contamination with Staph. aureus in hospital since home-born infants are not so frequently colonised (Ludlam,1953, Edmunds, Elias-Jones, Forfar and Balf,1955) .

Differentiation of Strains of Staphylococcus aureus

Gram-positive cocci occurring in clusters and growing on laboratory media to form relatively large, opaque colonies, can be recovered from a large number and variety of situations. For long they have been known to be associated with putrid processes outside and inside the body and their arrangement in clusters enables them to be distinguished from gram-positive cocci occurring in chains (Billroth, 1874). Ogston studied the organisms responsible /

responsible for suppuration and reported that the most frequently present in lesions were micrococci, either in chains or irregular clusters (Ogston,1880,1881).

Ogston recognised that these two groups of organisms, although appearing together in some cases, were generally found in association with a more or less distinct pathology. The first group was the chain form, or streptococcus of Billroth, and the second, with cells arranged in clumps or clusters was given the name Staphylococcus, from the Greek word for a bunch of grapes which they seemed to resemble (Ogston,1883).

At first differentiation of these cocci occurring in clusters was not seriously attempted. It was known that micrococci from the air and water could be injected with impunity into the tissues but on the analogy of Koch and Pasteur's work with Bacillus anthracis, the possibility of enhancement to pathogenic status of these harmless organisms could not be excluded. In fact Ogston went as far as to state ".... my conviction is that the common micrococci that exist around and on us .....are one and the same with the virulent cocci that cause inflammation".

However some differences in the staphylococci occurring in human infections were noted. One such important difference was reported by Verneuil (1880) in a paper entitled "de la suppuration orangée " in which he says it was "....associated with a dangerous form of blood poisoning and may relate to a strongly pigmented staphylococcus". Other colours, chiefly orange, were described by various investigators when examining pus associated with coccal infection, yellow and red. Ogston attempted to culture his organism in whole chick eggs by infecting albumen and noted that in some growth was whitish, in others yellow, and these colours were

to /

to a certain extent correlated with the clinical picture.

The recognition of differences in colour of artificial cultures by Ogston (1881) allowed the differentiation of white and yellow pigmented strains which were called, Staph. aureus and Staph. albus, and later of cocci producing other colours such as Staph. citreus. Rosenbach (1884) adopted Ogston's nomenclature for these organisms and with advances in cultural technique, which had rapidly taken place, was able to differentiate several varieties, and to these he gave names which described both source and colour on culture. Thus he gave trinomials, Staph. pyogenes aureus and Staph. epidermidis albus which have the advantage of being fully descriptive.

However this was too limited a scheme for distinguishing strains for epidemiological studies, and furthermore variation in the colour of growth occurred spotaneously in the cultures of many strains quite apart from differences due to cultural techniques.

Successes in the differentiation of strains of coliform organisms by means of noting their activity against defined chemical substrates led to a similar investigation of Staphylococci, Andrewes and Gordon, 1905) but in spite of exhaustive series of tests these reactions did not prove to be of very great value, partly due to the small number of differences in chemical reactions between the strains used and partly to the variability of those reactions that /

that did differ. In 1903 Gordon claimed to be able to differentiate staphylococci on the basis of cultural tests, and in 1905 Andrewes and Gordon divided staphylococci into 4 species on the basis of different biochemical tests. Ultimately most of these tests were eliminated and most staphylococci were defined on the basis of their pigment production, liquefaction of gelatin and fermentation of mannitol.

Other investigators such as Cumming and Cumming (1913) found these biochemical tests unreliable, especially as the results were not reproducible when repeated on the same strains several months later. It was thus already obvious that several features which served to distinguish individual strains of staphylococci were so slightly correlated with other attributes of the organism that they were unlikely to possess much taxonomic utility (Gibson,1948). Cumming and Cumming (1913) excluded micrococci occurring in tetrads and packets of eight, small colony strains and gelatin non-liquefiers. Those that remained had opaque colonies, 70 per cent of which were yellow and 30 per cent were culturally white or grey in colour. Subdivision of these remaining organisms was attempted by a selection of the more important biochemical tests, and finally on the basis of the fermentation of maltose and mannite. Five groups were recognised as under : /

Group	Action on Maltose	Mannite	Gelatin
1	-	-	+
2	-	+	+
3	+	-	+
4	+	+	+
5	+	+	-

Much variation in colour on culture within these groups was noted.

Thus some 25 years ago staphylococci were still distinguished on the basis of colony colour into Staph. albus, Staph. aureus and Staph. citreus. The majority of the strains isolated from pathogenic lesions gave some degree of pigmentation and Staph. aureus became synonymous with pathogenic staphylococcus ; Staph. albus being much less frequently isolated from lesions, and very commonly from other sources was believed to be non-pathogenic.

The early biochemical classification in a modified form is still utilised by some workers who characterise pathogenic strains as those fermenting mannitol and liquefying gelatin. A more recent attempt to improve this method of classifiation was that of Gibson and Abdel-Malik (1948) who have discarded what are considered to be the more useless tests and have admitted /

admitted to an essentially continuous series of strains, although 3 main divisions of cocci are recognised. At one end of the scale we have the pathogenic staphylococcus and at the other end thermoduric saprophytes. Thus;

Group I (Gibson) consists of Gram positive cocci, relatively sensitive to heat; fermenting glucose and forming acetoin and hydrolysing arginine. Mainly parasitic.

Group II Obligate aerobes which do not ferment sugars.

Group III Thermoduric sugar fermenters which appear to be strict saprophytes and may be sub-divided into two groups by their action on glycerol.

The differentiation of Staphylococci from various sources is necessary to the medical bacteriologist to distinguish between pathogenic and non-pathogenic strains; the epidemiological study of staphylococcal infections requires a much more detailed differentiation because of the ubiquity of organisms with similar morphological and cultural characters. The differentiation into pathogenic and non-pathogenic strains by virtue of colour on culture, or the fermentation of mannitol and liquefaction of gelatin was not satisfactory owing to the spontaneous variation in these characters which could occur in culture.

Loeb (1903) first showed that culture fluids of Staph. aureus were capable of coagulating goose plasma. Much (1908) repeated this /

this and showed that staphylococci from pathological material could coagulate human and rabbit plasma. This can be correlated with the clumping of these organisms in plasma due to the deposition of fibrin on their surfaces and is quite distinct from agglutinin activity. This early observation has been frequently confirmed and the power to coagulate plasma, usually of human or rabbit origin has been found to correlate very closely with probable pathogenicity, that is recovery of the strain from pathological material (Cruickshank,1937). There is also a fairly good correlation with the colour on culture, most strains with yellow or golden-yellow pigment producing coagulase and few strains with white, grey, red or lemon pigment producing the enzyme. White strains producing coagulase are usually regarded as variants of yellow pigmented strains.

In view of the close correlation between pathogenicity and coagulase production it has been suggested that strains producing the enzyme be called Staphylococcus pyogenes irrespective of colour. This has certain advantages which are not invalidated by the classification of Bergey which places staphylococcus within the genus Micrococcus (Cowan,1951). The term staphylococcus has precedence.

The term Staph. pyogenes is fully descriptive of a clumping coccus with the power of producing pus, and the binomial may be expanded to describe the colour of the strain if desired, thus Staph. pyogens var aureus /

Staph. pyogens var aureus or albus. In spite of this the term Staph. aureus persists by custom and is still used by many bacteriologists and most clinicians to indicate coagulase-positive staphylococci, with or without the production of yellow or orange pigment.

Thus using a technically simple test of plasma coagulation it is possible to differentiate potentially pathogenic staphylococci from those that are non-pathogenic and this has been extremely useful from the point of view of laboratory diagnosis, satisfying the immediate requirements of the diagnostic bacteriologist. However a much more detailed differentiation of the staphylococci is required for epidemiological study of staphylococcal infections. Attempts to do this were first made by Julianelle and Wieghard (1934) in distinguishing staphylococci by antigenic analysis. They separated Group agglutinins and more specific capsular polysaccharides from staphylococci derived from various sources and were able to distinguish two main groups corresponding to the pathogenic, coagulase positive organisms and the non-pathogenic, coagulase negative strains. Further subdivision of the pathogens was possible on the basis of their different capsular polysaccharides, but its usefulness in practice was limited by a degree of cross-reaction. Thus once again the degree of differentiation of strains although helpful and reliable was not of great epidemiological assistance. Much further differentiation of strains similar in cultural and biochemical characters was still required. The initial work in differentiation strains on a serological basis was followed up by many workers so that 3 groups of staphylococci could /

could be distinguished by agglutination tests with antisera, prepared in rabbits against known strains of Staph. aureus (Cowan 1938; 1939). These reactions were quite specific and the results when used were of considerable epidemiological value. (Hobbs 1944,1948; Allison and Hobbs,1947). Further subdivision of these sub-groups into types was attempted by absorbing sera but this was only moderately successful owing to the large amount of cross-agglutination, even with highly absorbed sera. At most 9 types, 3 in each sub-group could be distinguished by Cowan (1939) with the probability that there were probably many more but whose recognition was prevented by the technical limitations of the method. However the technique of serological typing of strains of staphylococcus has been applied in many investigations of the epidemiology of staphylococcal infections. (Cowan,1938,39; Hobbs, Carruthers and Gough,1947; Devenish and Miles,1939; Gillespie, Devenish and Cowan,1939; Oeding 1952,1954; Brodie, Somerville Wilson,1956.

Bacteriophage Typing of Staphylococcus aureus

A large proportion of strains of Staphylococcus aureus are lysogenic. Fisk (1942a) showed by cross-culture techniques that 19 of 43 strains carried phage. Among these he found 5 cultures which lysed spontaneously on storage. The phages isolated from these strains were fairly specific for the strains carrying them, acting on very few other strains.

The Development of Phage Typing Methods /

The Development of Phage Typing Methods

Following Burnet's observations on the relation between phage susceptibility and antigenic structure (Burnet and Lush 1935) workers in Germany used specific Salmonella phages for quick diagnosis (Sonnenschein,1927; Schmidt,1931). The first comprehensive phage typing method was introduced by Craigie and Yen (1938), who adapted a particular phage, specific for the Vi of Salmonella typhi to demonstrate specific types of antigen. Felix and Callow (1943) extended the method to S. paratyphi B in the same manner.

By similar techniques phage-typing has been evolved for various other bacterial species where serological, or other methods of differentiation were not sufficient, for example for Salmonellae, Pseudomonas and Staphylococcus.

The first attempt to classify Staph. aureus into types by means of bacteriophage was by Williams and Timmins (1938) using Burnet's strong phages Au_1 ; Au_2 ; Au_3 ; Au_4. (Burnet and Lush, 1935) and lysis in broth as their criterion. In this way they were able to distinguish 6 types by their pattern of lysis.

Fisk (1942b) laid the foundation of the present-day method when he isolated a series of 27 phages by picking plaques which had developed after spot inocula of several strains had been made on an agar plate seeded with a single strain. Each strain in turn was used as the basal strain. The areas showing phage action were picked off and suspended in distilled water, the lysogenic and susceptible strains identified, and the suspensions passaged several /

several times until confluent lysis of the susceptible strains was obtained. The final suspension was sterilised with 'zephiran' to give a concentration of 1/50,000 to 1/100,000. Using these phage preparations Fisk was able to classify 44 out of 95 strains into 37 groups.

Wilson and Atkinson (1945) isolated 7 phages from lysogenic strains by Fisk's cross-culture technique and prepared 11 more by growing the original phage in the presence of an insusceptible strain----", a variant being produced in much the same way as with Craigies' typhoid phages". The phages obtained were propagated in broth by adding the supernatant fluid obtained after centrifugation into a young broth culture of a susceptible strain until confluent lysis was obtained. Purification of the phages was carried out by picking a single plaque, replating and propagating as before until a high titre filtrate was obtained. The highest dilution showing confluent lysis on its propagation strain was used as the test dilution. A method was thus devised suitable for routine use by which 14 'types' were distinguished. Eleven of the phages were apparently type specific but the other three had wider host ranges. Confluent lysis only was considered.

Wahl and Lapeyre-Mesignac (1950) proposed a new method of recognising 'types' based on a division of the typing phages into major and minor. They used the 16 original phages and three new ones, 29A, 42D and 42E of Wilson and Atkinson, and a phage 68 isolated by themselves. Their major phages were 68, 52, 52A, 3C and 47A. Lysis by one or more of these phages, irrespective of degree determined to which of the 5 groups the strain belonged. Various sub-groups were defined according to the reaction of the minor phages. The major groups showed some correlation with the

serological groups of Cowan (1939) but this was not absolute.

In an investigation into the serological classification of Staph. aureus, Hobbs (1948) compared her serological groups with those defined by bacteriophage typing methods. Group I (29/52), Group II (3/A/ 3B/3C/51) and Group III (6/7/47) corresponded roughly to Cowan's groups I,II and III respectively (Wahl's corresponding groups were 68/52/52A ; 3C; and 68/47A.). There were many cross-reactions however with the additional types suggested by Christie and Keogh (1940) and four other types suggested by herself e.g. CK5, serologically related to Cowan's type I, was phage type 29/52, but her type 2 was lysed strongly by 3A, although it had little or no serological relation to C2. A third of 259 strains were untypable by phage methods but all were typable by agglutination reactions.

Hobbs concluded that there are a few specific types of staphylococci around which are grouped various numbers of sub-types, varying slightly in their antigenic structure, some of which can be recognised by their pattern of agglutination reactions. The same was true of bacteriophage typing which shows fairly close correlation with well-defined groups, but not always with well-defined types.

In a more recent investigation into the bacteriophage typing method Williams and Rippon (1952) stress that the phages in use at present /

present are not of the specific nature of Vi phages in Salmonellae, possibly because they have not all been adapted from a single parent phage. It follows that phage typing cannot differentiate specific types of Staph. aureus, but it is undoubtedly of value in determining the identity or non-identity of strains thought to be the same. These workers also criticise the method of reporting of Wilson and Atkinson, and Wahl and Lapeyre-Mesignac, and advise that all strong reactions be used, for using confluent lysis only 34 out of 100 random strains were typable, and 73 were typable using all strong reactions. A difficulty arises however with the increased complexity of phage lytic patterns. Eighty-two distinct patterns were observed in 229 strains using all strong reactions and 132 patterns in 295 strains when lesser degrees of lysis were considered.

Three broad groups of phages were apparent, and phage being found commonly to act in association with other members of the same group, but rarely with members of other groups. These groups included the following phages :

Group I 29:29A: 42B; 31: 44: 52: 52A; 73; 79; 80.

Group II 3A; 3B; 3C; 51:55:71.

Group III 6:7:42B; 42E;47:47A;47B;47C;54;70;75; 76;77.

These were similar to the groups described by Wilson and Atkinson, and Hobbs and Smith. The divisions are not well defined however and there is much overlapping of reactions. Although the phage /

phage patterns of two cultures thought to be the same were rarely identical, duplicate tests of a single culture showed less variation than duplicate cultures of a single source. This latter variation is assumed to be partly due to variations in typing on different days, since differences in medium have some effect and also to a real variation occurring in populations in their natural habitat. Attempts to distinguish between these two sources of variation were unsuccessful.

Complete identity of pattern was highest with duplicate tests of a single culture and the loss or gain of weak reactions was common in all tests. Loss or gain of more than one strong reaction was not observed in duplicate tests and was highest with sequential cultures from one source.

A number of untypable strains were observed, the larger number again being in the multiple and sequential cultures from the one site. The testing of these strains with undiluted phage filtrates led to the conclusion that the variations were due to two factors, namely a mixture of typable individuals in the population, and also to some extent a variation in sensitivity among the population. This degree of variation varied with the phage groups, being greatest with the 6/7/47 group. Groups 3A and 29 were roughly similar, but Gp.29 showed the greatest number of typable and non-typable mixtures, perhaps because the phages in this group are the most exacting nutritionally.

On /

On the basis of these results Williams and Rippon suggest that phage patterns showing a difference of more than one strong reaction when tested on the same day indicate a different population, but that a single strong reaction gained or lost may mean a different population in the 3A or 29/52 groups. Differences occurring on different days must be interpreted with more caution. The typing of a standard culture each day may help to interpret results.

In the present investigation extensive use of the phage typing method has been made to identify strains of Staph. aureus and study the distribution of the strains within and between the different population groups examined. The stability and reproducibility of the bacteriophage patterns of individual strains has also been examined and a study of the relation of phage pattern to antibiotic susceptibility made.

ANTIBIOTIC SUSCEPTIBILITY OF STAPHYLOCOCCUS AUREUS.

The problem of the susceptibility of Staph. aureus to antibiotics is probably of greater importance than with any other bacterial 'species' and the high incidence of antibiotic-resistant strains in hospital infections has made this a problem of pressing importance. The majority of publications on resistance to antibiotics deal directly or indirectly with the resistance of Staph. aureus to penicillin and the other antibiotics. No other species has shown such powers of adaptation, not only to penicillin, but successfully to each other antibiotic as it has been introduced.

In recent years the susceptibility of numerous series of staphylococci to penicillin and other antibiotics has been reported. The majority of these reports have concerned hospital staphylococci but a few have referred to the susceptibility of strains isolated from individuals among the general population.

As penicillin was the first antibiotic introduced on a large scale the earlier reports were concerned almost exclusively with the incidence of strains of Staph. aureus resistant to this antibiotic. The dramatic and beneficial effect of penicillin on serious staphylococcal infections made the appearance of refractory strains all the more striking and disappointing. However as the use of antibiotic was severely restricted during the years 1941-45, the number /

number of reports of less-susceptible organisms were few and did not indicate a large number of resistant strains. As soon as liberal supplies were available and the profession was able to use penicillin in ever increasing doses, the results of treatment of staphylococcal infection became steadily worse and the proportion of strains reported as resistant to the antibiotic by *in vitro* tests increased at an alarming rate. Thus Rammelkamp and Maxon (1942) first reported an increase in resistance of 4 strains of *Staph. aureus* in the course of penicillin therapy. At the same time they were able to show that 29 strains tested *in vitro* adapt themselves to grow in higher concentrations of the antibiotic. *Spink and Vivino*(1944) reported 11 per cent of their strains resistant to 0.4 -0.8 units/ml. of penicillin, and by 1945 Plough reported that 40 per cent of his strains were resistant to 0.5 units/ml. Other reports were those of Gallardo (1944) with 12 per cent resistant, Hartley and Bowie (1946) with 9.4 per cent and Bondi and Dietz (1945), Finland et al (1950), Florey et al (1949) and Duguid (1946). An increasing number of reports followed and it has been suggested by Needham and Nichols (1953) that as far as penicillin-resistant strains are concerned in hospital a state of equilorium has been reached so that generally speaking 75 per cent of hospital strains of *Staph. aureus* in medically advanced countries are penicillin-resistant.

The proportion of antibiotic resistant strains has remained lower /

lower in the general community (Gohar,1951; Thomson and Schwabacher, 1951; Vogelsang,1953, Lowden,1954, Wise, Cranny and Spink,1954; Fairbrother,1956; Markham,1958) and in out-patients (Voureka and Hughes,1949; Markham,1958 than in hospital. Few antibiotic-resistant strains are found in primitive populations who have not come into contact with antibiotics (Hoffs, Wisseman and Whelan,1954, Rountree,1956; Markham,1959).

Cairns and Summers (1950) showed that open infections acquired antibiotic- resistant Staph. aureus in proportion to the length of stay in hospital, though this was unrelated to antibiotic therapy (Summers,1952; Lepper, Dowling, Jackson and Hirsch,1953). Nurses and patients acquire antibiotic resistant strains in hospital (Goldberg and Masterton,1956; Clarke,1957; Knight and White,1956; Williams et al., 1959) after some weeks stay but shed them to a certain extent when they leave (Dowling, Lepper and Jackson,1953).

The most striking figures illustrating the increase in the incidence of penicillin-resistant strains in hospital are those of Barber (Barber 1947(a) and (b); Barber and Rozwadowska-Dowzento 1948) referring to 3 successive years in the same hospital; in 1946 the proportion of penicillin-resistant strains examined was 12.5 per cent; in 1947 it was 59 percent and in 1948, 83 per cent. Her evidence shows that a few resistant strains increased by simple selection and there was not an actual increase in the number of resistant strains.

However /

However it has often been suggested that staphylococci have acquired resistance from contact with penicillin in man and arisen during treatment (North and Christie,1945,1946; Plough, 1945; Blair, Carr and Buchman,1946, North, Christie and Rank,1946). Such changes might be due to the following events.

1: Reinfection during treatment with a penicillin-resistant strain not previously present may occur, and removal of the original, penicillin-sensitive strain, by the antibiotic would occur.

2: the selection of a spontaneous mutant, resistant to penicillin and present in the original bacterial population.

3: selection of an induced mutant by the antibiotic.

4: Adaptation of the bacterial population to the antibiotic on the part of all the individuals in the population.

Rake, McKee, Lamre and Houck (1944) however were unable to induce a rise in the <u>in vivo</u> resistance during serial passage of <u>Staph. aureus</u> in mice treated with sub-curative doses of the antibiotic. North, Christie and Rank (1946) also made an attempt by injecting staphylococci subcutaneously, along with antibiotic intraperitoneally, but no increase in resistance developed, though there was a change in serological character. Nevertheless there is the impression that penicillin-resistant staphylococci appear more often after therapy than not, (Nolan and Fleischer,1958) particularly in chronic infections, though Cairns and Summers (1950) found no relationship between resistance /

resistance and treatment in in-patients, but did find that out-patients increased their chance of acquiring penicillin-resistant staphylococci when open wounds were exposed to cross-infection (Summers,1952).

Blair, Carr and Buchman,(1946) found increases of many thousand-fold in resistance to penicillin during the treatment of chronic osteo-myelitis wounds.

However experimental conditions *in vivo* are such that in most cases it is impossible to study pure cultures of micro-organisms let alone individual cells. In addition to the possible presence of more than one strain, cross infection during treatment must be taken into account. Therefore the following explanations may be put forward.

(1) A true acquisition of penicillin-resistance such as occurs in *Bacillus cereus*, which produces penicillinase as a true adaptive enzyme.

(2) Simple selection of pemicillinase producing strains (Barber,1947a) following - mutation (Spink and Ferris,1947) independent of the antibiotic.

Much work has been carried out to examine antibiotic-resistance developed *in vitro* where it is easier to observe and control the experimental conditions. These studies have indicated that penicillin-resistant strains of *Staph. aureus* may be of two types. The most frequently described is that which takes place by a series of small increases in resistance when the micro-organism is /

is cultured in increasing concentrations of the antibiotic. Abraham (1941) reported resistance developing *in vitro* repeated sub-culture in broth containing sub-inhibitory concentrations of penicillin. Similar methods have been used many times to train sensitive strains to grow in the presence of penicillin (Rake et al,1944; Chain and Duthie,1945: Blair et al 1946; Spink and Ferris 1947, Gale,1947a; Bellamy and Klimek, 1948a; Gale and Rodwell,1949, Monnier and Schcenbach,1951) and resistant variants growing in as much as 6,000 units per ml. have been obtained Gale (1947b) Gale 1949, Bellamy and Kilmek,1948a, Kilmek, Cavallito and Bailley,1948). Monnier and Schoenbach (1951) found no relationship between the degree of acquired resistance and initial susceptibility. The rate of acquired resistance and the degree of resistance varies from strain to strain . Graessle and Frost (1946) had a variation ranging from 4 to 15000 times while Kilmek et al (1948) produced an organism 80,000 times as resistant as the parent strain by serial subculture.

The second type of penicillin-resistance described *in vitro* occurs when there is a sudden and great increase in resistance and the resistant variant is found to produce penicillinase. A great increase in resistance of this kind was obtained by Kirby (1945) after a single culture in the presence of penicillin, but most frequently penicillinase-producing variants seem to be present as a minority of the original culture. Sybalski (1953) however /

however reported the isolation of penicillinase producing mutants by a gradient-plate technique from a culture previously shown not to contain such variants.

From laboratory studies it is apparent that the first type of variant differs in many of its biological features from the parent strain and from resistant staphylococci isolated from patients. Growth is relatively poor, the colonies are small and lack opacity and often pigment, and there may be much variation of colonial appearance even within the same culture. Coagulase production is often weak or completely lost and the gram-staining reaction may be converted from positive to negative. (Gale, 1949) and a rod shaped cell produced. Resistant strains with these characters are not described arising in vivo although 'G' variants (Swingle, 1935) are similar.

The second type of penicillin-resistant organism however possess all the biological characters of the parent penicillin-sensitive culture and are resistant to the antibiotic because of its destruction by the enzyme, and they are similar to the penicillin-resistant organisms that are isolated from patients and carriers.

It has been shown by Spink et al. that penicillin-resistant strains of Staph. aureus isolated from lesions vary in their power of producing penicillinase and also in the quality of the enzyme that they produce. Previous experience of penicillin is an important factor in determining the quantity and quality of the enzyme.

For this reason Parker (1946) and Barber (1947) found that variation in the inoculum of these strains in in vitro susceptibility tests had a marked effect on the results. This has led to considerable confusion in the correlation of laboratory susceptibility tests and the results of antibiotic therapy.

Most investigators are in general agreement that penicillin-sensitive Staph. aureus, that is strains not producing penicillinase, have a similar degree of sensitivity to penicillin. Thus any appreciable decrease in susceptibility in an in vitro test should indicate potential penicillinase production and corresponding possibility of clinical resistance; certainly such strains should be regarded as resistant by definition.

The introduction of Streptomycin with its relatively high activity against Staph. aureus whether penicillin resistant or sensitive, raised the hope of clinicians that penicillin-resistance need not any longer be regarded as a drawback or worry; the new antibiotic when it became available was administered to many cases of staphylococcal infection. Unfortunately the same developments took place as had occurred with penicillin, but at an even quicker rate, so that within a remarkably short time reports of streptomycin-resistant strains were received from various hospitals. In some the relative numbers of streptomycin-resistant strains approached that of penicillin-resistant strains. The unforunate pharmacological effects of streptomycin however started to restrict the /

the indiscriminate use of this antibiotic and it is true to say that streptomycin resistance has not therefore come to be regarded as a great problem in relation to Staph. aureus. This attitude was encouraged by the timely arrival of Chloramphenicol (Chloromycetin) which, although not so highly active against Staph. aureus on a weight for weight basis as penicillin or streptomycin, was sufficiently so to treat cases of infection. It was not long before chloramphenicol resistant strains began to be reported and their numbers in hospital environments became appreciable. This number fell as the consumption of chloramphenicol dropped when it was reported that this antibiotic had an adverse report on haemopoetic tissue and might be related to an increased incidence of leukaemia. The drop in consumption was also accelerated by the introduction of chlortetracycline (aureomycin) and later oxyteracycline (terramycin). Along with tetracycline (achromycin) these drugs have been effective in staphylococcal infections and they are active against strains of Staph. aureus irrespective of the susceptibility of these strains to other antibiotics. However strains of staphylococci resistant to all of the tetracyclines have emerged and almost invariably a strain resistant to one tetracycline antibiotic is resistant to the others due to cross-resistance. At the present time there is a fairly high incidence of tetracycline resistant strains in hospitals.

The /

The more recent introduction of erythromycin has seen a repetition of the now familiar story and erythromycin-resistant strains of staphylococci have appeared wherever the drug has been extensively used in the treatment of infections.

The few reports concerning Staph. aureus in the general community have borne out the contention that the results penicillin therapy are still good in the majority of staphylococcal infections occurring in general practice. Thus Elwood (1951a) found that on first attendance at hospital all the carrier strains of patients were penicillin-sensitive. Rountree and Barbour (1951) found that among nurses entering training school, only 4 per cent of the nasal carriers yielded penicillin-resistant strains, and Burrows et al (1955) found that 19 of 21 strains of Staph. aureus from cases of sycosis barbae were penicillin sensitive.

However some investigators have suggested that the proportion of penicillin-resistant strains of Staph. aureus in the general community is rising and this may be more rapid in the USA than elsewhere. For example Dowling et al (1953) found that 30 per cent of nasal carrier strains from householders were penicillin-resistant, but Rountree et al (1954) found that 13.4 per cent of strains isolated from blood donors were penicillin resistant compared with 6 per cent in 1951, though it is true that some of these persons had been hospitalised.

Reversal of Penicillin resistance. There is considerable disagreement about the permanency of penicillin resistance which has been acquired by training and is of the non-penicillinase producing type. Most investigators have found penicillin resistance in Staph. aureus to be gradually lost during repeated sub-cultures in the absence of the antibiotic (Spink et al,1944; Blair et al, 1946; Eriksen,1949; Graessie and Frost,1946, Spink and Ferris 1947; Klimek et al. 1948). The original degree of susceptibility was not always regained even after a long time (Chain and Duthie,1945; Demerec,1945a,b.) and Demerec regarded it as a permanent acquisition, though the degree of resistance induced in his experiments was small.

Barber (1949) has shown that naturally occurring penicillin-resistant staphylococci produce penicillin-resistant mutants on repeated subculture in penicillin-free medium and pointed out the variation in the behaviour of individual cells in a bacterial population to the antibiotic and suggested that a few penicillinase producing cocci may protect penicillin sensitive cocci from the action of penicillin. Luria (1946) concluded that penicillinase production need not be associated with the penicillin resistance of the individual cells which may themselves be highly sensitive to the antibiotic. Gilson and Parker(1948) found the average resistance of penicillinase-producing strains to be 250 times greater than sensitive strains when a large inoculum was used but only 8 times if the inoculum was small.

The /

The present investigation has examined the antibiotic-susceptibility of many strains of Staph. aureus from different sources and its relationship to susceptibility to bacteriophages. The association of antibiotic-resistant strains to antibiotic therapy and the use of antibiotics has been examined in detail and this has allowed some speculation on the reasons for emergence and spread of antibiotic resistant staphylococci.

METHODS USED TO ISOLATE AND IDENTIFY STAPHYLOCOCCUS AUREUS.

1. THE SAMPLING OF SURFACES FOR STAPHYLOCOCCI

The surfaces of the body and fomites were sampled with dry sterile cotton-wool swabs after experiments had been carried out to confirm that dry swabs were preferable to wet swabs for the collection of Staphylococcus aureus (Rubbo and Benjamin, 1951).

Experiment - to measure the survival of Staph. aureus on cotton-wool swabs.

A person known to be a persistent nasal carrier of Staph. aureus was used as a source of the organism. Dry cotton-wool swabs (A) and similar swabs soaked in sterile water (B) and in serum (C) were used to sample the anterior nares of this carrier. The swabs were rotated over the lining of the anterior nares and rubbed thoroughly over the surface of culture plates. After incubation comparative colony counts were made.

To rule out sampling errors the swabs were used in the following order. At the first swabbing an (A) was used to sample the nose first, followed by (B) and then (C). At the second swabbing (B) was used first, followed by (C) and then (A); at the third (C) was used first, followed by (A) and finally (B).

Further experiments tested the time of survival of the organisms on the swabs by allowing the swabs to lie at room temperature for varying lengths of time before applying them to culture plates. Comparative colony counts were again made.

From the results shown in Table 1 it is clear that Staph. aureus survived for a longer time on the dry swabs than on/

STAPHYLOCOCCAL COLONY COUNTS ON CULTURE PLATES				
Time elapsed between sampling and culturing		Dry swab 'A'	Water swab 'B'	Serum swab 'C'
Hours	0 - 1	+++	+++	+++
	2 - 6	+++	+++	+++
	6 - 12	+++	+++	+++
	12 - 24	++	++	++
Days	1 - 2	++	++	++
	3 - 4	500	300	350
	4 - 6	150	200	200
	6 - 8	75	20	30
	8 - 10	40	10	15

Recovery of Staphylococcus from Wet and Dry Cotton-wool Swabs after Sampling the Same Source.

TABLE 1.

on the swabs moistened with either water or serum, though the difference was not great until the interval between sampling and culturing was greater than 48 - 72 hours. However, a greater drawback to the wet swab was the frequent overgrowth by other species of micro-organism, such as coliforms, when the interval between sampling and culturing the swabs was prolonged. This agrees with the finding (Daranyi, 1925.) that Staph. aureus is well adapted to withstand drying. Also other species of micro-organism which are likely to be sampled from the source, may be more liable to die on the dry swab so that if the interval between sampling and culturing is prolonged, Staph. aureus will have a distinct survival advantage.

For these reasons dry swabs were used throughout the following experimental work.

The anterior nares of individuals were sampled by rotating the swabs six times within the nostrils so that the first three-quarters of an inch was covered; swabs were similarly rotated or stroked over other selected sites such as the palms and backs of the hands, fingers, face, surfaces of the chest and back, axillae, umbilicus, perineum and groin. Within as short a time as possible the swabs were rubbed over the surface of culture medium so that the maximum numbers of organisms were transferred.

Swabs were also used to sample lesions which were producing exudate. In some cases exudate was submitted for bacteriological examination.

2. CULTURAL METHODS/

2. CULTURAL METHODS

Staphylococcus aureus grows readily on most laboratory culture media, and blood agar and nutrient agar (Mackie and McCartney, 1957) are suitable for its isolation in most cases. However, in much of the experimental work reported in this thesis the organism was present in relatively small numbers in the material examined, or was present with a large number of other species and so a medium with some differential and selective properties was desirable.

Nutrient agar containing milk was recommended for the study of staphylococci by Christie and Keogh (1940) following the original description by Fushita and Yoshioka, 1938, that milk allowed good differentiation of staphylococcal pigments. At first there was some difficulty in preparing this medium due to the breakdown of milk-sugars during sterilisation which resulted in caramelisation and a brown colour of the medium. When this happened the colour of the colonies could not be recognised so easily and growth of the organisms was poor. To avoid this the medium was prepared in the following ways:-

(a) Skimmed milk was sterilised once by steaming in a Koch steamer and added in the ratio of one part to two of 3 % nutrient agar base at 55°C. This was thoroughly mixed and cast into Petri dishes which were stored at 4°C. for up to one week.

(b) Alternatively, satisfactory results were obtained by adding commercially available homogenised and 'sterilised' milk to 3 % nutrient agar base.

This/

This 33 % milk-agar medium was found excellent for the growth and ready recognition of colonies of staphylococci which appeared as smooth, opaque, convex discs about 2 mm. in diameter within 24 hours. To allow for the slower pigment production in some strains, the plates were allowed to stand at room temperature for a further 24 hours before final observations were made. Most of the other species of bacteria found on the sites that were examined were partially or completely inhibited on this medium, or grew colonies which were readily distinguished. Thus most streptococci and diphtheroid bacilli did not grow; colonies of Neisseria were small and translucent, and Escherichia and other coliform bacilli grew well to give large transparent colonies. Proteus was not inhibited.

This milk-agar medium was compared with other media commonly used for the isolation of Staph. aureus such as nutrient agar, nutrient agar containing 10 % blood and Fildes digest agar (Mackie and McCartney). Growth of the staphylococcus was equally rapid on these standard media and milk-agar but the differentiation between the various shades of yellow and white pigment was not so easily appreciated. Also the contrast between colonies of Neisseria, Diphtheroids and Yeasts on one hand and staphylococci on the other was not as marked on the standard media.

Enriched milk-agar medium

The growth of staphylococci on the standard milk-agar medium just described was enhanced by the addition of potato-extract or serum, or both.

(a) Potato-milk agar /

(a) Potato-milk agar

Potatoes were peeled and cut up into small pieces which were added to tap water in a flask and steamed for 1½ hours. This mixture was passed through fine muslin to make a uniform suspension of potato pulp and 10 % by volume of this pulp was added to 3 % nutrient agar base and stored.

Potato-milk agar was prepared by adding one volume of sterile milk to two volumes of potato-agar base.

The colonies of staphylococci grew to a larger size on this medium than on milk-agar and pigment production was much enhanced.

(b) Serum-milk agar

This was prepared by adding 10 % by volume of sterile serum to nutrient agar or potato-agar base at the same time as the milk. Growth of staphylococci was much enhanced and coagulase positive colonies were surrounded by a zone of partial clearing of the medium due to breakdown of the serum protein. The growth of streptococci and Haemophilus and some other more exacting species was better on this medium than on milk-agar.

More highly selective media

When very small numbers of Staph. aureus had to be isolated from among large populations of other bacteria, or in cases where rapidly growing contaminants were liable to overgrow the staphylococcus, more highly selective media than ordinary milk-agar were required.

A characteristic of the staphylococcus is its resistance/

resistance to high concentrations of sodium chloride so that most strains will grow in the presence of 12 - 15 %. Selective media were therefore prepared by adding 10 % sterile sodium chloride to various stock media -

1. Fildes Digest Broth.
2. Robertson's Meat-piece Broth.
3. Milk-agar.
4. Potato-milk agar.

The growth of most bacterial species encountered, with the exception of the Staphylococcus, was effectively prevented by the salt. The growth of all strains of staphylococci was also slower than in salt-free medium but full growth and colour differentiation took place within 4 days.

The prevention of mould contamination on culture plates.

Many culture plates required to be left at room temper-:ature for a few days before final observations were made and in some cases this allowed contaminating moulds (e.g. Mucor, Aspergillus, Penicillium, Gliocladium and Trichoderma) to overgrow the surfaces precluding proper examination and sub-:culture.

To overcome this difficulty anti-fungal antibiotic such as Mycostatin (Nystatin) was used. A solution of Mycostatin was prepared in methyl alcohol and diluted in sterile sodium citrate to give a final concentration of 1,000 units per ml. Over the surface of each culture plate 0.2 ml. of this solution was spread uniformly and allowed to dry prior to use.

Examination of cultures/

Examination of cultures

Primary cultures on milk-agar medium (or other solid media if used) were examined for staphylococcal colonies and an approximate count made using the following symbols:-

\+ = up to 20 colonies

++ = from 20 - 50 colonies.

+++ = numerous colonies.

++++ = confluent growth.

Primary cultures in fluid media (usually salt enrichment broth) were subcultured on to milk-agar medium and examined for staphylococcal colonies.

Maintenance of strains

Subcultures were made from single isolated colonies into nutrient broth and incubated for 18 - 20 hours. These cultures were used to test for coagulase and penicillinase production and susceptibility to bacteriophages and antibacter-:ial substances.

For short-term maintenance the broth cultures were inoculated on nutrient-agar slopes in $\frac{1}{4}$oz. screw-capped bottles. These were incubated for 24 hours and stored at room temper-:ature with the caps screwed tightly down. Subcultures from these agar cultures were made in nutrient broth as required.

For long-term maintenance cultures were freeze-dried. One-tenth of a millilitre of fluid broth culture was added to each freeze-drying tube (3" x 0.25") along with a sterile strip of filter paper marked with the identifying number of the strain. These tubes were placed in a vacuum dessicator

over/

over phosphorous pentoxide and the pressure slowly reduced to prevent excessive frothing of the liquid during the evolution of dissolved gases. When this was complete the pressure was reduced quickly with a high-vacuum pump, and freezing of the cultures took place within a few seconds. Pumping was continued for 30 - 45 minutes before sealing the dessicator and leaving the open tubes over phosphorous pentoxide for 24 hours. Following this the dessicator was opened and each tube was attached to the pump and sealed in vacuo.

Cultures were dried in quadruplicate so that reference to the original cultures could be made on several occasions if necessary.

3. Examination of strains of staphylococci.

The production of coagulase : Freshly isolated colonies of staphylococci were examined for coagulase production.

Lominski (1948) maintains that citrated plasma may coagulate with organisms other than staphylococci and so re - :commends the use of heparinised plasma. Coagulase-inhibiting substances are found in some specimens of plasma from healthy humans (Lominski, 1946) and it is clear that there are marked species variations in reaction to staphylokinase (Gerşheim, 1949).

For these reasons pooled rabbit plasma was used throughout the following experiments for coagulase tests.

The following techniques were used:-

(a) Slide method (Cadness-Graves, 1943).

Separate colonies of staphylococci were emulsified in drops of diluted and undiluted plasma on glass slides.

Spontaneous/

Spontaneous clumping of the suspension with clearing of the suspending liquid is believed to be due to coagulase as fibrin produced during the coagulation of the plasma adheres to the staphylococci binding masses of them together in macroscopic clumps.

A drawback to the test was the occurrence of minor degrees of clumping of the suspension without extensive clearing of the suspending fluid. In such cases it was difficult to decide whether the test was positive or negative. Many of the strains giving these doubtful reactions proved to be negative by other methods of estimating coagulase production (Table 2) and for this reason all strains were tested by the tube test.

(b) Tube test of Fisk (1940)

A colony, or in some cases several colonies of the growth on the primary culture, was emulsified in 0.5 ml. of rabbit plasma diluted 1 in 5 with physiological saline. The emulsion was incubated at 37°C. for 24 hours, but inspected at 4, 12 and 24 hours for coagulation. The production of visible clot was regarded as evidence of coagulase production. Alternatively, 2 drops of a broth culture of the test organism were added to 0.5 ml. of the diluted rabbit plasma.

Results of coagulase tests

The coagulase test was used to separate the strains of staphylococci isolated on primary culture into those that were coagulase positive, described as Staphylococcus aureus, and those that were coagulase negative, described as Staphylococcus albus, irrespective/

			Results of the 'Slide Test' Number of Strains		
Results of the 'Tube Test'.			Positive	Negative	Doubtful
			2290	702	458
Number of Strains	Positive	2071	1920	64	87
	Negative	1379	370	638	371

RESULTS OF TESTING 3450 STRAINS OF STAPHYLOCOCCI FOR COAGULASE PRODUCTION BY THE 'SLIDE' AND 'TUBE' TEST.

TABLE 2.

Pigment Produced on Culture	Number of Strains Examined	'Tube Test' Percentage Coagulase Positive	Coagulase Negative
YELLOW	2190	93	7
WHITE	1260	3	97

COAGULASE PRODUCTION BY YELLOW AND WHITE PIGMENTED STRAINS OF STAPHYLOCOCCI

TABLE 3.

irrespective of the pigment produced on culture.

The results of the present investigation show that (Table 3)

(1) not all strains producing yellow pigment produced coagulase.

(2) the number of white or grey pigmented strains producing coagulase was very small.

(3) a few coagulase negative strains were susceptible to some of the stock bacteriophages. In these cases we must assume that the coagulase test was at fault.

(4) a few antibiotic-resistant variants of antibiotic-sensitive parent cultures were poor coagulase producers or did not produce coagulase at all.

4: Determination of susceptibility to antibacterial substances.

These determinations were carried out by methods used in other forms of microbiological assay. The principles are shown in the accompanying figure (Fig. 1).

A concentration gradient of the antibacterial substance was prepared in a medium that was fully adequate to support the growth of the test organism which was added uniformly to the mixture. Following incubation, growth occurred in the medium where the concentrations of inhibitory substance was below the inhibitory level and ceased where this level was reached. This allowed assessment of the susceptibility of the micro-organism.

Two techniques were used in performing these tests - one using fluid medium and the other using solid medium. The essential difference between the two techniques was that the concentration gradient was continuous using solid media whilst

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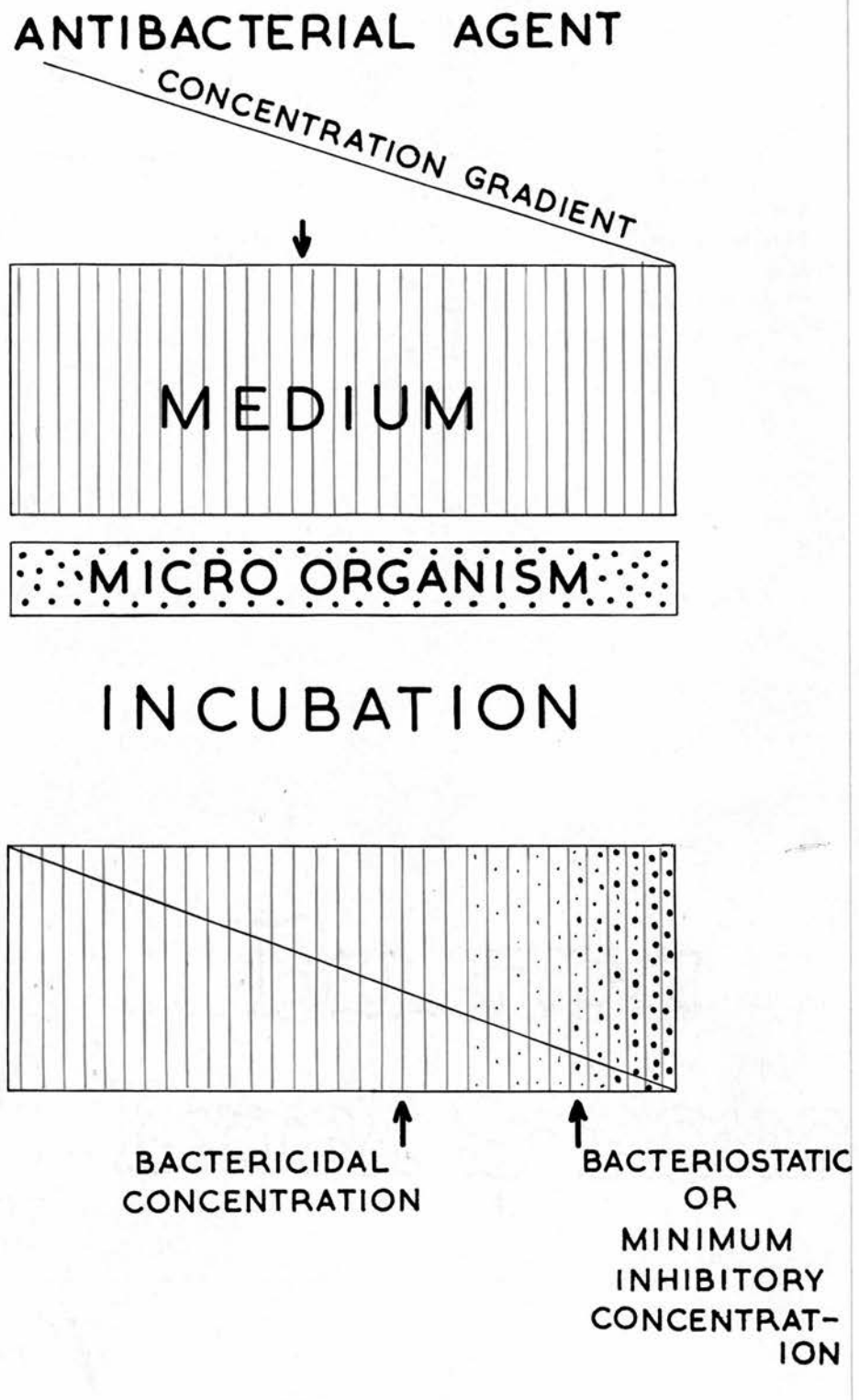


PRINCIPLES OF ANTIBACTERIAL SUSCEPTIBILITY TESTS

FIGURE 1.

it was discontinuous in the tests using fluid media.

Susceptibility tests using fluid media

The serial dilution tube test : This is the classical form of antibiotic susceptibility test in which a series of tubes containing nutrient medium and varying amounts of inhibitory agent were inoculated with the organism under test. Following incubation the tubes were examined for visible growth of the organism which was compared with that in a control tube containing no inhibitory agent. The amount of inhibitory substance in the first tube showing diminished growth was interpreted as the minimum inhibitory, or bacteriostatic, concentration (M.I.C.). To determine the bactericidal concentration it was necessary to subculture from the tubes showing little or no growth, into medium free of inhibitory agent, to ascertain the number of viable organisms remaining (Bigger, 1944). The concentration in the first tube with no surviving organisms was interpreted as the bactericidal concentration representing 100 % killing of the bacterial population.

In carrying out these tests the experimental conditions were identical in each tube and the only variable was the amount of inhibitory substance. The result is a figure representing the concentration which will inhibit the test organism under the *in vitro* experimental conditions of the test and its relationship to the amount required to inhibit the same organism *in vivo* is debatable. The value of the results of these tests was thus primarily comparative; a means of comparing the strains one against another, and of comparing individual strains at different/

different times. The accuracy of these tests must be measured in terms of the reproducibility of the results. For this reason it was most important to include replicate tests for each strain and a control test with a standard organism of known susceptibility with each batch of tests.

The outstanding drawback to the serial dilution test described above was the amount of time and material required to carry out a large number of tests. For this reason the alternative technique using solid medium and a continuous concentration gradient was adopted to carry out most of the susceptibility determinations reported in these experiments, as it can be adapted to use readily available laboratory apparatus and multiple tests can be carried out with great facility.

Susceptibility tests using solid media

The inhibitory agent may be incorporated in the test media in several ways but is usually held in a reservoir from which it diffuses through the agar to form a concentration gradient.

The reservoir may be a hole or gutter cut out of the agar medium and filled with the inhibitory agent in solution, or incorporated in agar; alternatively it may be placed inside a cylinder resting on the surface of the medium (Fleming, 1929; Foster and Woodruff, 1943; Abraham, 1941; Foster and Woodruff, 1944). This latter method has the advantage that a more uniform area of the medium is in contact with the antibacterial solution so that diffusion will be more regular. The same

advantages/

advantages are obtained by using absorbent paper containing the inhibitory agent in contact with the surface of the agar. The paper is easier to handle and makes unnecessary the care required in handling plates with cylinders full of fluid. The use of absorbent paper as a means of carrying antibacterial solutions on the surface of solid media was first suggested by Pope (1940), and a few years later was adopted by a number of investigators for antibiotic test purposes (Morley, 1945; Thomson, 1950; Kolmer, 1947, and Gould and Bowie, 1952). As a result it is now widely used and many modifications of the technique have been developed. Filter paper is the most convenient form of adsorbent paper likely to be uniform in quality so that pieces cut from the same grade of paper will absorb the same amount of fluid if they are of the same size. Extensive experiments were carried out with filter paper discs as reservoirs of antibiotic for diffusion into agar and these have become the basis of a disc diffusion test for determining the susceptibility of micro-organisms to antibiotics and antibacterial agents.

The disc diffusion test

This test depends upon the diffusion of the inhibitory substance from the disc outwards through the surrounding medium agar. Since the inhibitory agent is continuously diffusing, the concentration gradient will never be constant and the rate of growth of the micro-organism under test will therefore be important. The results of the test also depend upon factors influencing the diffusion of the inhibitory substance including the/

the pH of the medium, its thickness, the degree of hydration of the agar and the nature of dissolved substances in the agar gels. However if these factors are experimentally controlled the disc test can give results of a high standard of reproducibility, whilst having the great advantage of the ease with which the discs can be prepared and stored; a reasonable degree of accuracy due to the exact amount of fluid which each disc absorbs and the even and constant contact of the disc with the surface of the medium. An added advantage is the ease with which replicate tests can be set up, and the large number of different inhibitory agents which can be tested on the same plate.

Preparation of the discs

The grade of paper used was Whatman No. 1. Uniform areas of these papers were cut and found to weigh the same within small limits. When the edges of these pieces of paper were brought into contact with water each piece absorbed the same amount as shown by weighing before and after absorption.

A further test was carried out by dipping similar pieces of the filter paper into antibiotic solution. The saturated paper was then dried and cut up into smaller pieces of equal size; each piece was assayed for antibiotic and found to contain the same amount.

For experimental test purposes round discs were cut out of commercial sheets of filter paper fabric (Whatman No. 1 grade) with a hole puncher having a hole size of $\frac{1}{4}$ inch (6.3 mm.). The discs so produced were fractionally larger, having a mean diameter/

diameter of 6.34 mm. and varying insignificantly in size as long as the instrument was kept sharp and single thicknesses of paper were punched; punching of single sheets also ensured that the discs remained discrete so that they were more easily counted and handled.

After cutting, the discs were counted into lots of 100 and put into 1 oz. screw-capped wide-mouth 'Universal' containers. These bottles and their contents were sterilised in the hot-air oven for 1 hour at 140°C. Charring of the paper was avoided. The following experiment was carried out to find out how much liquid was taken up by the discs.

Experiment : One hundred discs were placed in 1.0 ml. of water in a small bottle. The discs were then removed individually and the residual fluid was weighed.

The results (Table 4) show that 75 % of the water was absorbed.

Preparation of solutions of antibacterial substances and impregnation of discs

The solutions were prepared by dissolving suitable crystalline salts of the various inhibitory substances in distilled water. The antibiotics used were commercial preparations of high standard used for clinical purposes and labelled with sufficient accuracy for the purposes of these tests. The other substances were either commercial products or trial substances obtained direct from the manufacturer.

As the discs were shown to absorb less than 0.01 ml. of water/

Bottle containing 100 discs	Weight of water remaining after removal of discs	Volume absorbed by each disc
Batch No. 1	290 mgms.	0.0071 ml.
" 2	260 "	0.0074 "
" 3	250 "	0.0075 "
" 4	280 "	0.0072 "
" 5	230 "	0.0077 "
Average	262 "	0.0074 "

UPTAKE OF WATER BY FILTER-PAPER DISCS

TABLE 4

of water, 1.0 ml. of the required antibacterial solution was added to each bottle of 100 discs. This saturated all the discs and a small amount of excess fluid remained. Thus the solutions of antibacterial substances were prepared to contain in each millilitre, 100 times the quantity required in each disc. The amounts in each disc were arbitrarily chosen to produce zones of inhibition of growth approximately 25 mm. in diameter with susceptible strains. The following substances were used in the experiments reported, and the amounts used in the test discs are given in the table (Table 5).

Recognition of the discs

The presence of several discs of identical appearance on a single culture plate made some means for the identification of the individual discs desirable. The most satisfactory solution to this problem was the use of coloured discs. Originally, Morley (1945) used blue and white blotting paper discs to distinguish between sulphonamides and penicillin on agar plates. The dyes used to tint blotting paper are cellulose fast and do not interfere with the growth of bacteria (Morley, 1945) and the present experiments indicated that they do not interfere with any of the antibiotics or antibacterial agents tested (Bowie and Gould, 1952). The following cotton dyes were used to colour the paper discs used in this work.

Aniline tolamine pink	(Clayton R)
Durazol yellow	(I.C.I. G.R. 200)
Chlorazol sky blue	(I.C.I. F.F. 200)
Durazol fast orange	(I.C.I. R. 150)
Durazol scarlet	(I.C.I. 4B.150)

and with them a wide range of colours was prepared by mixing (Table 6).

Antibacterial Agent	Concentration in solution for impregnating discs	Amount in each disc
	Mgm/ml.	ug.
Penicillin	0.1	1
Streptomycin	1	10
Chloramphenicol	2.5	25
Chlortetracycline	5	50
Oxytetracycline	1	10
Tetracycline	1	10
Erythromycin	1	10
Spiramycin	2	20
Neomycin	1	10
Novobiocin	1	10
Polymixin B	10000 units	100 units
Bacitracin	1	10
Sulphathiazole	25	250
Chlorhexidrene	5	50
Propamidine	25	250
Xanthacillin	5	50
Furadantin	2	20
"23082"	10	100
Viomycin	10	100

CONCENTRATIONS OF ANTIBIOTICS USED IN THE DISCS

TABLE 5.

ANTIBIOTIC	DYE OR DYE MIXTURE	COLOUR OF DISC
Penicillin	Red	Pink
Chloramphenicol	3 : 1 Yellow and blue	Green
Chlortetracycline	Yellow	Yellow
Oxytetracycline	1 : 1 Orange and scarlet	Terra-cotta
Tetracycline	Orange	Orange
Neomycin	4 : 1 Orange and blue	Olive green
Erythromycin	1 : 1 : 8 Orange, blue and red	Puce
Polymixin	4 : 1 Red and blue	Mauve
Bacitracin	3 : 1 : 1 Yellow, blue and orange	Brown
	1 : 1 : 1 Orange, blue and red	Black
Streptomycin	-	White

COLOURS FOR ANTIBACTERIAL DISCS

TABLE 6.

Experiments showed that discs impregnated with solutions containing 2.5 mgm. per ml. had a sufficiently characteristic colour. Sterile dye solutions of the required concentration were therefore added to the solutions of antibacterial substances prior to impregnating the discs. In the case of the basic antibiotics streptomycin, neomycin and viomycin, and alkaline solutions of the sulphonamides, precipitation of the dyes occurred so these solutions were not coloured. The discs of streptomycin were left uncoloured and those for the other agents mentioned were coloured before impregnating with the antibacterial solution.

The impregnated discs were used wet and the bottle containing them was stored in the refrigerator at 4°C. without a great loss of potency for at least 3 months (Fig. 2). Before use the bottles were shaken to distribute the discs around the container walls so that they were more easily picked up. The discs were transferred to the inoculated plates with a pair of fine pointed forceps which were carefully flamed between each transfer to avoid contamination of the discs.

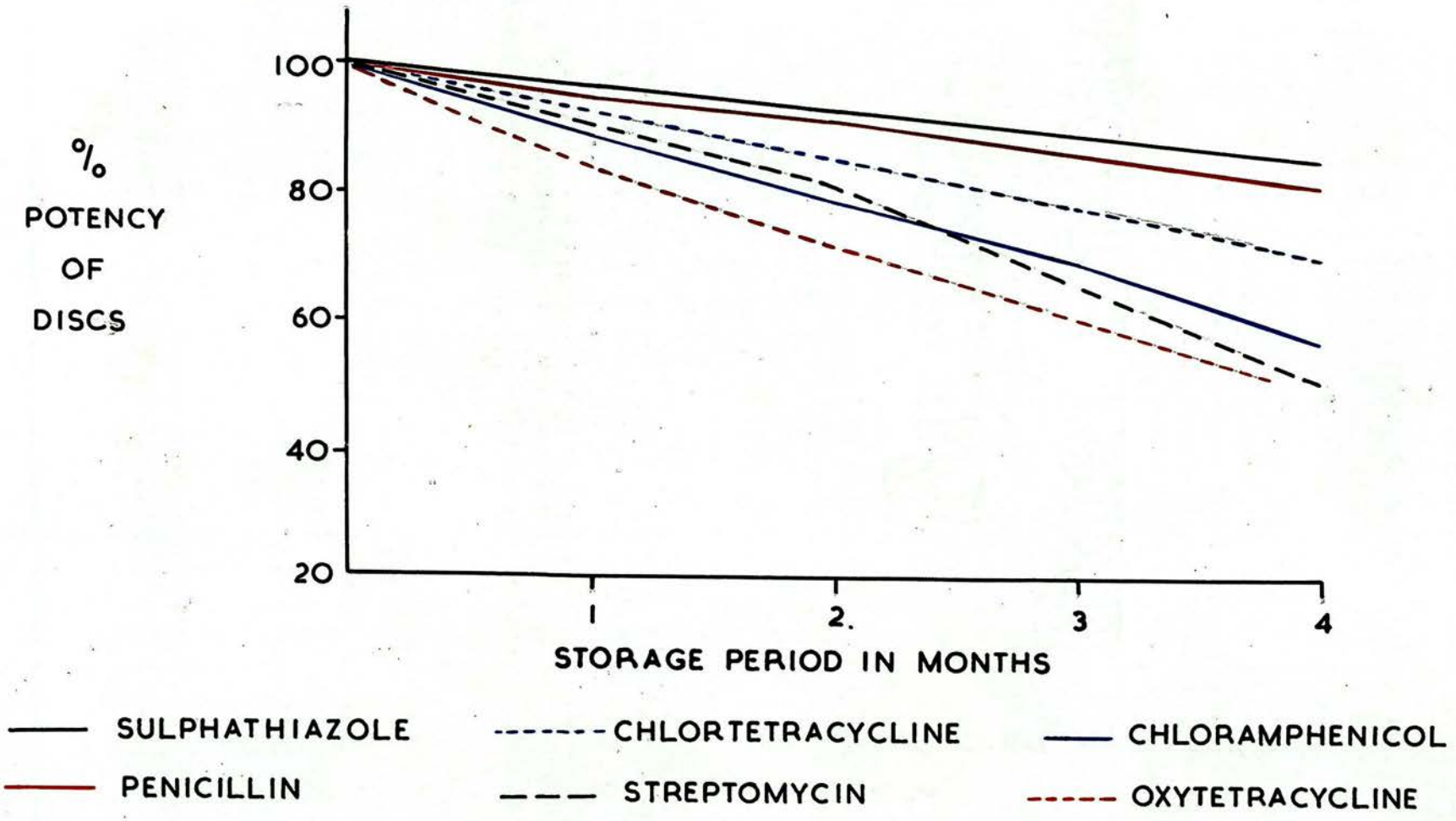

%
POTENCY
OF
DISCS
100
80
60
40
20
1
2.
3
4
STORAGE PERIOD IN MONTHS
SULPHATHIAZOLE
CHLORTETRACYCLINE
CHLORAMPHENICOL
PENICILLIN
STREPTOMYCIN
OXYTETRACYCLINE

FIGURE 2.

Technique of the test

1. The discs used were all of uniform size, from the same weight of paper and quality.
2. One millilitre of antibacterial solution was added to 100 discs and these were used within one month of preparation.
3. Standard Pyrex Petri dishes of 7.5 cm. diameter were cast with 10 ml. nutrient agar medium so that the thickness of the medium was 3.2 mm.
4. The medium was surface sown with overnight broth cultures of the organisms to be tested, containing approximately 100 million cells per ml. The plates were dried for 30 minutes at 37°C. and the discs applied.
5. The zones of inhibition of growth were read after 20 hours incubation at 37°C.

The zones of inhibition of growth

With susceptible organisms there were several zones apparent around the disc. They were:- (Figures 3 and 4)

(a) the zone of complete inhibition of growth. Prolonged incubation did not result in any change in the diameter of this zone.

(b) a zone of delayed growth where the colonies were smaller. Microscopic examination of the organism growing in this area showed morphological changes in the cells (Duguid, 1946; Ingram, 1951).

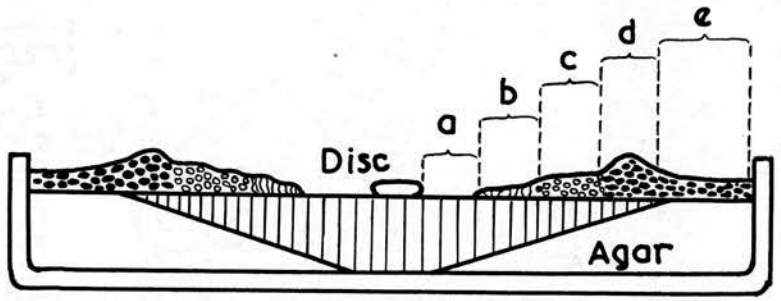
(c) the zone of lysis which appears after the initial period of growth. Microscopic examination reveals ghost cells within this zone.

(d) a zone of apparent stimulation of growth which was, in most cases, due to excess food material available at the boundary where autolysis of cells in the lytic zone was occurring.

(e) finally, outside the inhibitory zones, the region of normal growth. All of these zones were not always apparent, depending on the susceptibility of the organism and the time of incubation. With antibacterial agents other than penicillin the zone of lysis rarely occurred.

Measurement of the zones of inhibition was best made with a pair of dividers, using in a strong light reflected from the surface of the medium. The diameter of the zone was measured in preference to the radius or the distance from the edge of the disc to the boundary of the zone of inhibition.

Reproducibility of zones of inhibition/



(a) Zone of complete inhibition of growth.
(b) Zone of bacteriostasis.
(c) Zone of lysis.
(d) Zone of increased ('stimuulated)growth.
(e) Zone of normal growth.

FIGURE 3.

Zones of inhibition of growth of a penicillin-sensitive staphylococcus producing no penicillinase. The disc contains 1 unit of penicillin. From within outwards there are a zone of complete inhibition, a bacteriostatic zone, a broad zone of lysis, an ill-defined zone of stimulation and finally normal growth at the periphery of the plate.

FIGURE 4.

Reproducibility of zones of inhibition

The following experiment was carried out to measure the degree of reproducibility that could be expected with the discs as prepared.

One thousands discs impregnated with 1 ug. of penicillin were taken from stock and used under the test conditions described to inhibit the standard staphylococcus. The results are shown in Table 7.

The standard organism

As a control and for the preparation of standard graphs for each antibiotic a strain of Staph. aureus (Oxford S5) was used. This organism had been subcultured for many years in the laboratory. It had a phage susceptibility pattern 52/52A, and the following inhibitory concentrations of the various antibiotics, using serial tube dilution test. (Table 8).

The standard staphylococcus was maintained on nutrient agar slopes and subcultured into nutrient broth for 18 hours at 37°C. prior to use. The resulting culture was diluted to contain approximately 100 million organisms per ml.

Preparation of the standard graphs

The preparation of the standard graphs for penicillin will be described in detail. The curves for the other antibiotics were prepared in a similar manner and do not require to be individually described.

Solutions of crystalline sodium Penicillin G in distilled/

Number of tests	Mean diameter of zones of inhibition	Standard deviation	Standard error of mean
1,000	30.13 mm.	1.075 mm.	0.034

ZONES OF INHIBITION OF GROWTH OF STANDARD STAPHYLOCOCCUS IN A THOUSAND CONSECUTIVE TESTS WITH DISCS CONTAINING PENICILLIN.

TABLE 7.

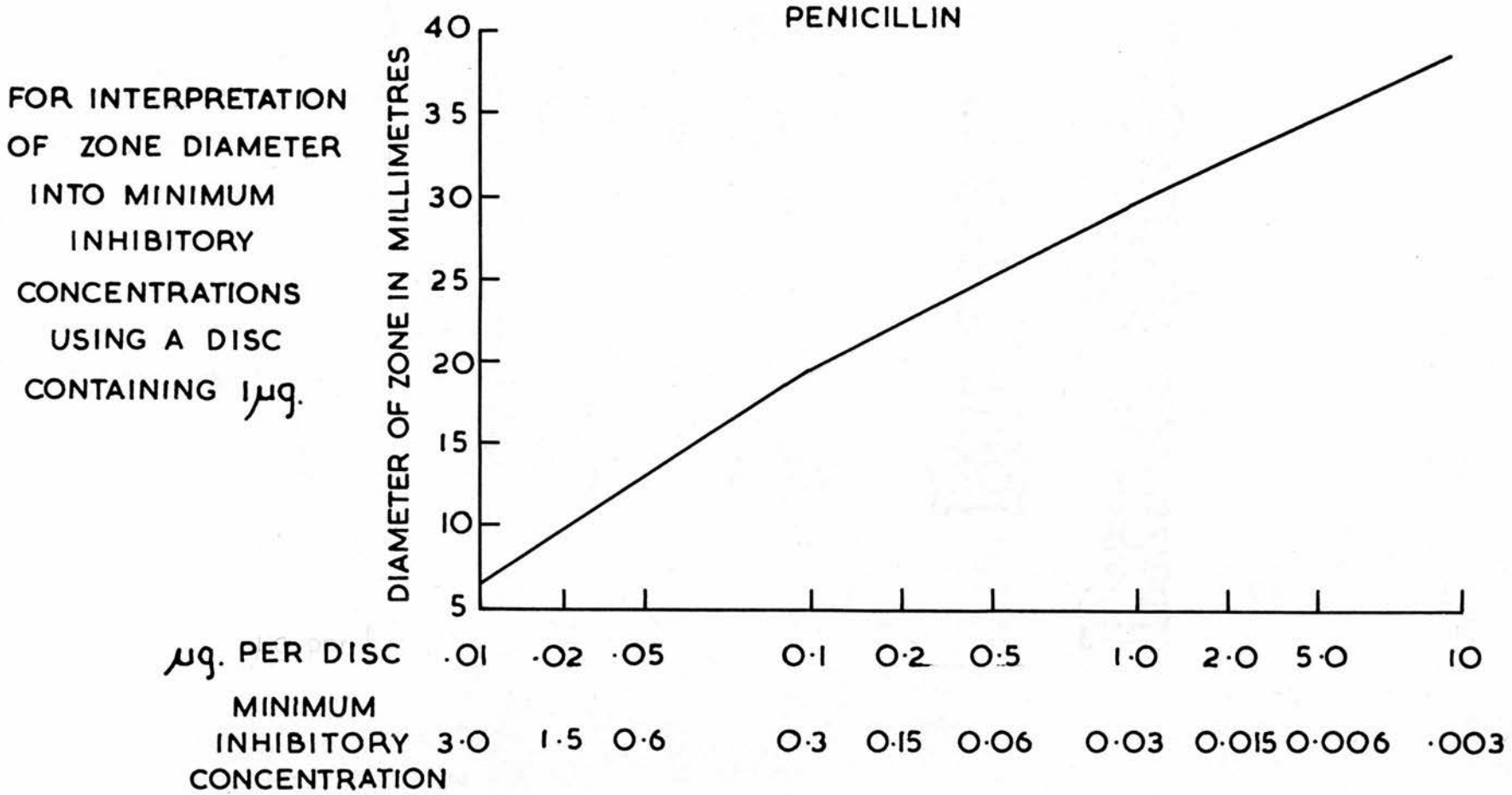
Antibiotic	M.I.C. (Bacteriostatic)	Bactericidal concentration
Penicillin	0.02 ug/ml.	0.05 ug/ml.
Streptomycin	0.25 "	1.25 "
Chloramphenicol	1.5 "	2.5 "
Chlortetracycline	0.25 "	2.5 "
Oxytetracycline	0.25 "	5.0 "
Tetracycline	0.5 "	5.0 "
Erythromycin	0.2 "	2.0 "
Novobiocin	1.0 "	2.5 "

SUSCEPTIBILITY OF STANDARD STAPHYLOCOCCUS TO COMMON ANTIBIOTICS.

TABLE 8.

distilled water were prepared to give the following range of discs containing 0.001, 0.005, 0.01, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10 and 20 ug. The discs were impregnated as previously described. Petri dishes containing nutrient agar or blood agar were surface sown with a broth culture of the standard staphylococcus containing 100 million organisms per ml. flooded on to the medium with a Pasteur pipette. The excess culture fluid was removed and the surface of the medium dried in the incubator for 30 minutes. The impregnated discs were applied carefully to the inoculated surface of the plates, centrally when single, or eccentrically when there was more than one on each plate. The plates were now incubated for 18 hours at 37°C. when the resulting zones of inhibition of growth (Table 8) were measured and plotted on graph paper (Fig. 5) against the amount of antibiotic in the disc. In each experiment a minimum of 10 replicates were set up for each concentration.

The antibacterial substance, in this case penicillin, is continually released from the paper disc from the moment of contact with the surface of the agar medium until equilibrium between the concentration of antibiotic in the agar and disc is reached. Diffusion through the agar takes place at a rate depending on the solubility of the antibacterial substance and its diffusion coefficient. The result is, that at any given time there is a concentration gradient from the disc outward, which is diagramatically represented in Fig. 3. The micro-organisms/



STANDARD GRAPH FOR PENICILLIN

FIGURE 5.

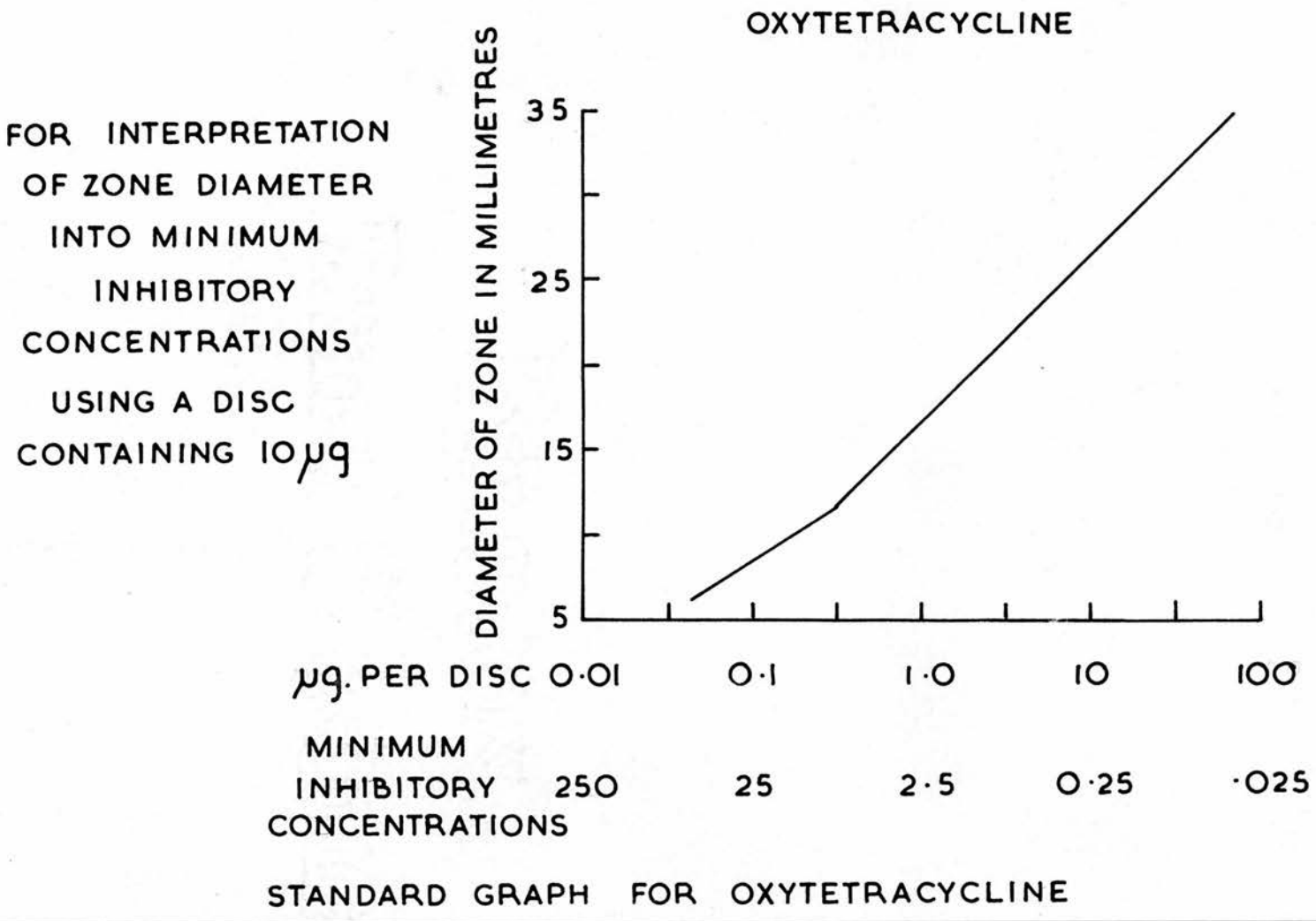
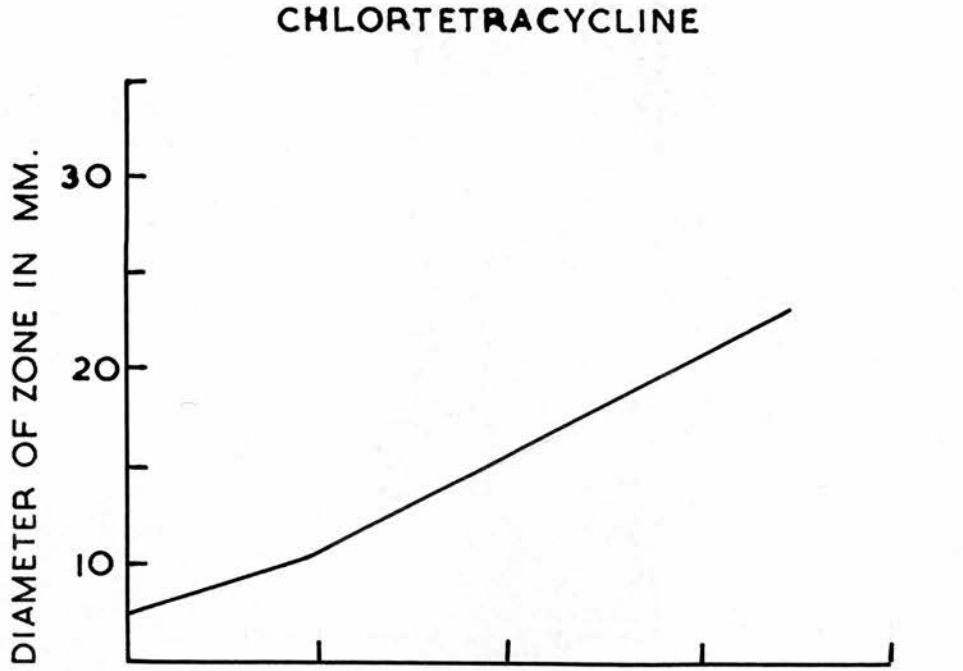

OXYTETRACYCLINE
FOR INTERPRETATION OF ZONE DIAMETER INTO MINIMUM INHIBITORY CONCENTRATIONS USING A DISC CONTAINING 10µg
DIAMETER OF ZONE IN MILLIMETRES
35
25
15
5
µg. PER DISC 0·01 0·1 1·0 10 100
MINIMUM INHIBITORY CONCENTRATIONS 250 25 2·5 0·25 ·025
STANDARD GRAPH FOR OXYTETRACYCLINE

FIGURE 5a.

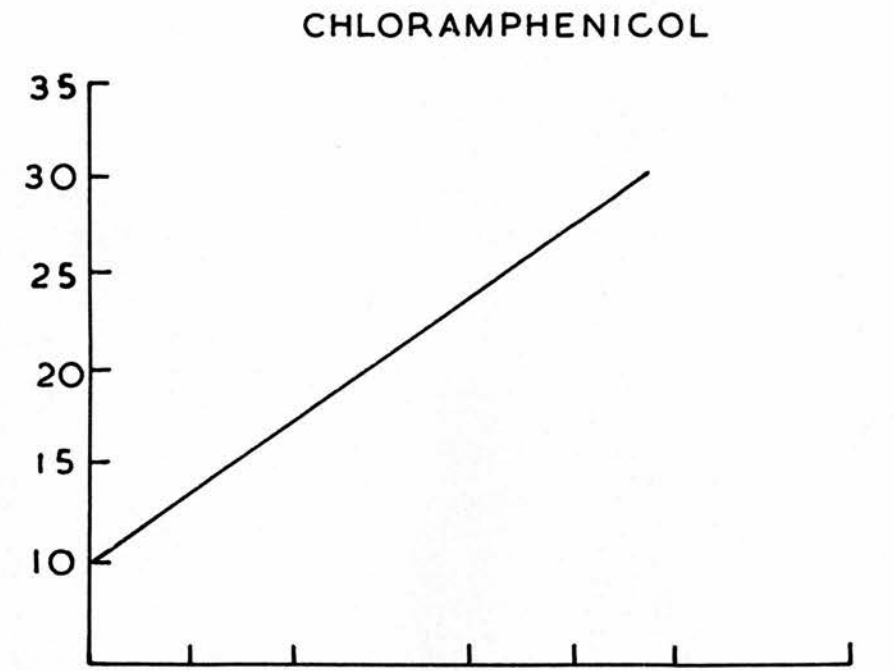
FOR INTERPRETATION OF ZONE DIAMETER INTO MINIMUM INHIBITORY CONCENTRATIONS USING A DISC CONTAINING 50 µg



STANDARD GRAPH FOR CHLORTETRACYCLINE

FIGURE 5b.

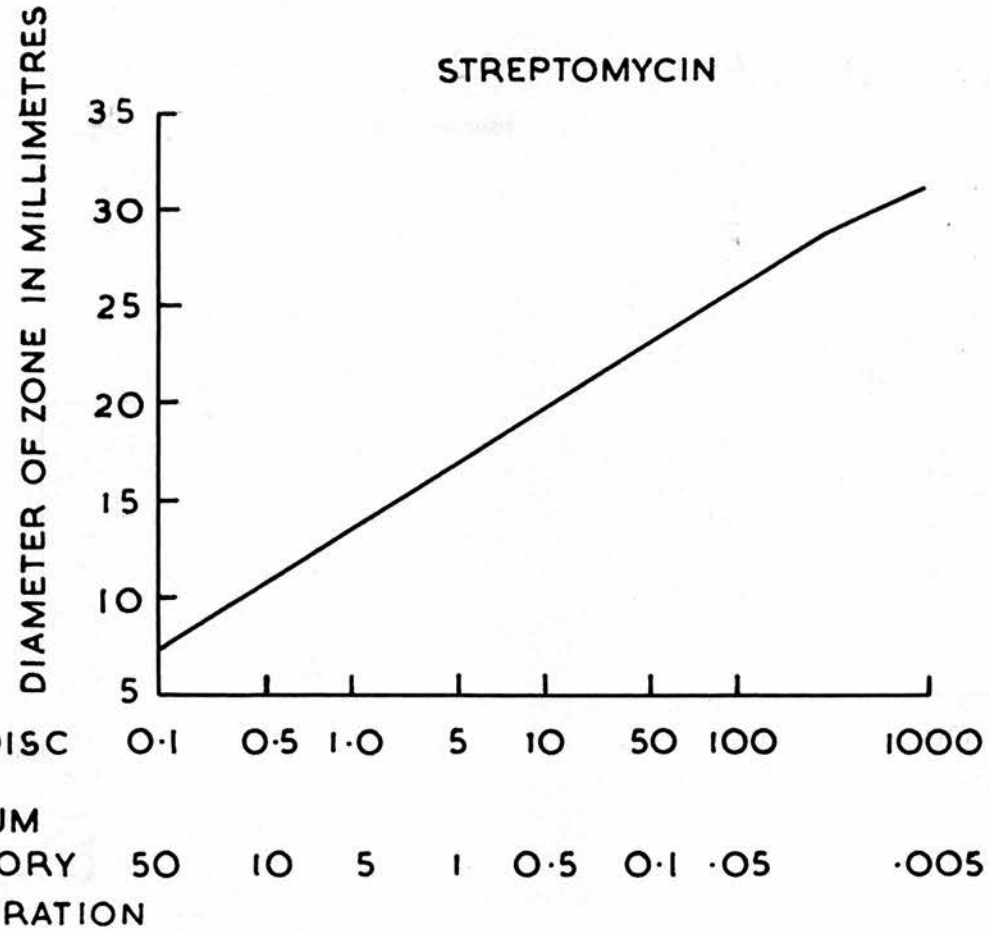
FOR INTERPRETATION OF ZONE DIAMETER INTO MINIMUM INHIBITORY CONCENTRATIONS USING A DISC CONTAINING 25μg.



STANDARD GRAPH FOR CHLORAMPHENICOL

Figure 5c.

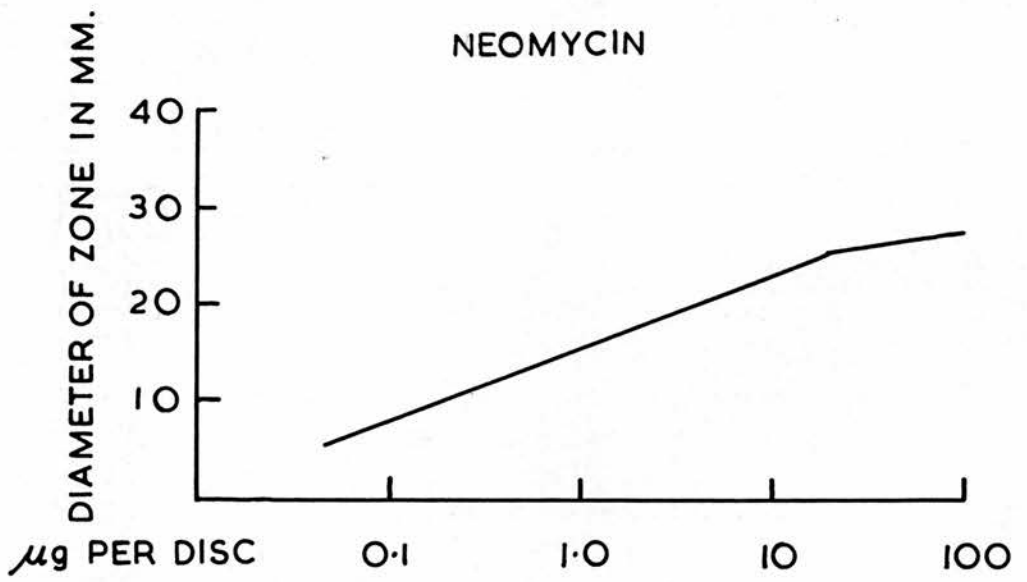
FOR INTERPRETATION OF ZONE DIAMETER INTO MINIMUM INHIBITORY CONCENTRATIONS USING A DISC CONTAINING 10 µg.



STANDARD GRAPH FOR STREPTOMYCIN

FIGURE 5d.

FOR INTERPRETATION OF ZONE DIAMETER INTO MINIMUM INHIBITORY CONCENTRATIONS USING A DISC CONTAINING 10 μg



STANDARD GRAPH FOR NEOMYCIN

FIGURE 5e.

micro-organisms growing on the surface of the medium, or alter-:natively throughout the medium if they have been mixed with the medium in a 'pour' plate, will be subjected to this variation in concentration and will not grow nearer the discs than the point where the inhibitory concentration is present. The result is a zone of inhibition of growth, and since the discs may be regarded as a point source, the zone is circular.

Diffusion of the antibiotic must take place before the logarithmic phase of growth if clear-cut zones of inhibition are to result. If conditions are maintained constant in all tests, the rate and extent of diffusion will be constant and identical zones of inhibition of growth will be obtained with the same organism. If the organisms to be tested are comparable to the standard organism in their rate of growth, the zones of inhibit-:ion may be compared with those of the standard organism.

In the experiments reported in this thesis conditions of test were maintained constant throughout, and as all the strains examined were Staph. aureus were cultivated under similar conditions.

Quantitative interpretation of the disc diffusion test

As with the serial tube dilution test the chief value of this test lies in the comparison of the susceptibility of various strains and thus it is desirable to be able to relate the zones of inhibition of growth to the amounts of antibacterial agent required for inhibition of growth.

Under standard experimental conditions the size of the zone/

zone of inhibition with any test organism depends on the susceptibility of that organism. A zone of the same diameter as that obtained with the standard organism signifies that the concentration required to inhibit the test organism is the same as that required to inhibit the standard organism. If the zone of inhibition with the test organism differs from that obtained with the standard organism, the test organism requires a greater or lesser concentration for inhibition depending on whether the zone is smaller or greater. Thus the susceptibility (M.I.C.) of any test organism may be calculated from the prepared graphs by reference to the amount of antibiotic required to inhibit the standard organism to the same diameter. The susceptibility of the standard Staph. aureus is known, therefore the calculation of the susceptibility of the test organism is as follows:-

$$\frac{\text{Amount per disc required to inhibit test organism}}{\text{Amount per disc required to inhibit the standard organism to the same degree}} \times \text{Susceptibility of standard organism}$$

For all tests, discs containing the same amount of antibacterial agent were used, therefore the amount used to inhibit the test organism was constant. The amount required to inhibit the standard organism is obtained from the prepared graphs as in the following example.

Suppose the diameter of the zone of inhibition of a test organism using a 1 µg. penicillin disc is 13 mm; the graph for penicillin/

penicillin indicates that a disc containing 0.05 ug. penicillin will inhibit the standard organism to 13 mm., therefore the sensitivity of the test organism is -

$$\frac{1}{0.05} \times 0.03 = 0.6 \text{ ug/ml.}$$

The abscissae of the graphs were modified to read directly in minimum inhibitory concentrations as shown.

5 The measurement of penicillinase production

All strains less susceptible to penicillin than the standard strain of Staph. aureus were tested for the production of the enzyme penicillinase. The following methods were used:-

1. Modified after Gots (1945) to detect extracellular penicill-:inase. Petri dishes were cast with 10 ml. of 2 % nutrient agar containing 1.5 ug. of penicillin and 0.1 ml. of a 24 hour broth culture of a penicillin-sensitive staphylococcus. The strains of Staph. aureus to be tested were streaked over the surface of the agar and the plates incubated overnight. Penicillinase production was shown by the growth of the penicillin susceptible organism sown in the agar under the surface growth of the test organism. Three controls were set up with each test - (a) a plate of nutrient agar sown with the susceptible staphylococcus; (b) nutrient agar containing the susceptible staphylococcus and penicillin and (c) nutrient agar containing the susceptible staphylococcus and penicillin streaked with a solution of penicillinase.

2. Some penicillin-resistant strains apparently producing no penicillinase/

penicillinase in the above test were examined for intracellular penicillinase. The test organism was inoculated into broth and the tube sealed with paraffin wax. After several days' incubation many of the cells were autolysed; the broth was centrifuged and the supernatant tested for penicillinase by streaking over the surface of an agar plate containing a sensit-:ive organism and penicillin.

3. The appearance of the zone of inhibition of growth around a penicillin disc : The typical appearance of the zones of a fully susceptible organism around a penicillin disc have been described. The characteristic feature is lysis (Fleming, 1929) and no strains showing a ring of lysis in the susceptibility test were found to produce penicillinase. (Figure, 4).

Many strains were observed which gave a zone of inhibition of growth around penicillin discs, varying in diameter from 7 - 30 mm. but lacking any zone of lysis so that there was a sharp margin to the line of growth. Fig 6 Microscopic examination of the staphylococci at the margin of growth showed that they had normal morphology. If the inoculum used was diluted so as to allow only discrete colonies to grow, the zone of inhibition increased in size and the colonies at the edge of the zone of inhibition were normal in their size and other cultural characters. There were no stunted or lysed colonies (Ingram, 1952). (Figure,7).

All the strains, showing these features, that were tested were shown to produce penicillinase; therefore these appearances/

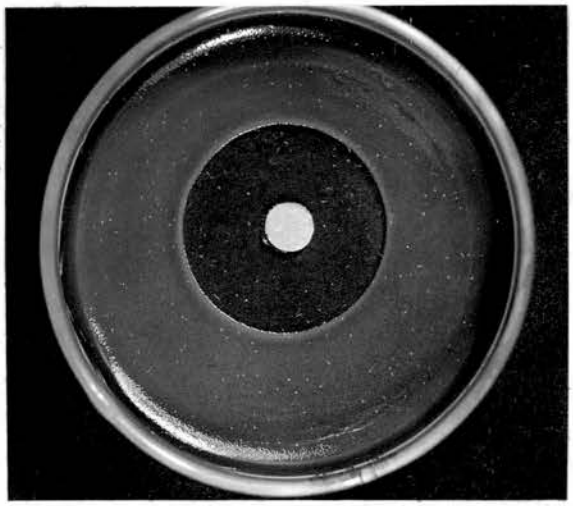
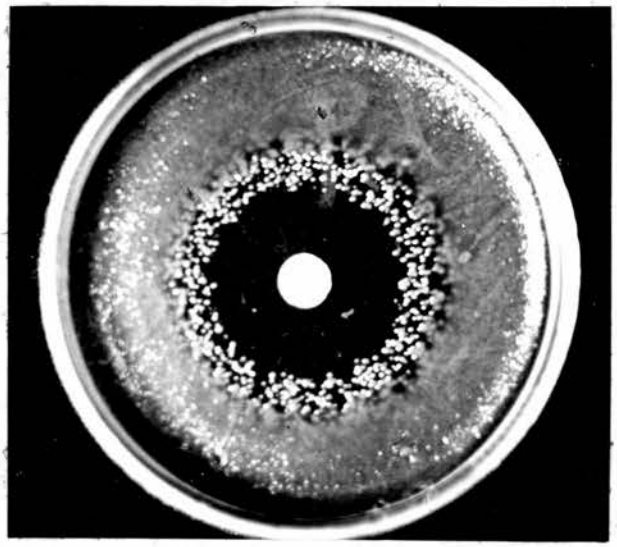


FIGURE _6

Zone of inhibition of growth of a penicillinase producing staphylococcus. The disc contains 1 unit of penicillin per ml. Note the wide zone of complete inhibition of growth with a sharp margin and the abscence of a lytic zone.

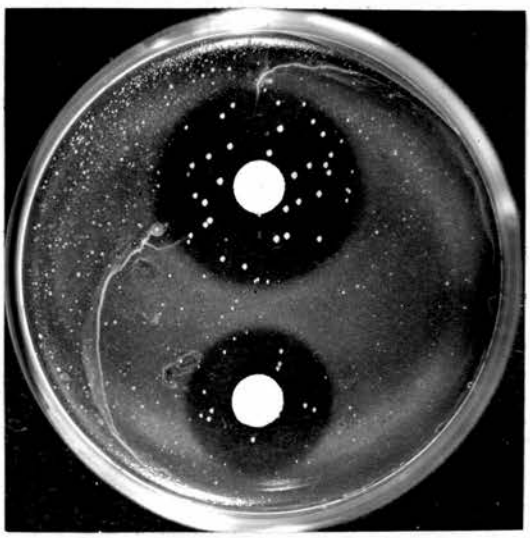


Zones of inhibition of growth of a penicillinase producing staphylococcus. The disc contains 10 units of penicillin. There is a zone of complete inhibition of growth and a ring of discrete colonies within this zone of inhibition of the majority of the bacterial population. These colonies have arisen from variants in the population having a higher resistance to penicillin.

FIGURE 7.

appearances on the susceptibility-test plate may be taken as presumptive evidence of the production of penicillinase. Such organisms are potentially resistant irrespective of the zone of inhibition of growth, and the amount required to inhibit growth depends very much on the size of inoculum.

The reason for the sharply demarcated line of growth and the absence of lysis is that the penicillin diffuses rapidly out from the disc and, depending on the amount of penicillinase actually present or being produced by the inoculum, inhibits and kills the staphylococcal cells, for these cells, free of penicillinase, are susceptible to the antibiotic (Spink, 1956). If little or no penicillinase is present in the inoculum the zone of inhibition approaches the size found with sensitive organisms since the diffusion of the antibiotic occurs during the lag period of growth. However, if the strain is capable of producing penicillinase, production of the enzyme increases as the lag phase passes and the penicillin in the medium is destroyed. This prevents any further antibacterial action on the more peripheral cells. No growth occurs within the zone of inhibition although the penicillin is destroyed by counter diffusion of the penicillinase as all the staphylococci have been killed; if some have survived, colonies within the zone of inhibition do grow. (Figure, 8).



Zones of inhibition of growth of a strain of Staph.aureus produced by penicillin (1 unit,upper disc) and streptomycin (10 ug., lower disc). A small number of cocci of much greater resistance than the remainder of the population have survived to produce the colonies within the zones of inhibition.

FIGURE 8.

6: THE SAMPLING OF AIR-BORNE DUST FOR STAPHYLOCOCCI

The air-borne dust of various sites was sampled by using a modified 'sieve sampler' after De Buy and Crisp (1944). This instrument sucked 25 cubic feet of air per minute through 400 holes uniformly distributed over a brass plate, on to the surface of a culture plate held $\frac{1}{8}$ inch away. Most of the dust particles in the air were trapped on the surface of the plate, the efficiency being about 60 % for particles of the size 2 to 5 microns.

After a suitable sampling period the culture plates (Sampling Plates) were incubated at 37°C. for 24 - 48 hours and colony counts made.

The sampling plates were usually cast with milk-agar treated with mycostatin and the agar concentration increased to 3 %. In heavily contaminated environments the sampling plates were exposed for 15 - 60 seconds (corresponding to $6\frac{1}{4}$ - 25 cubic feet) so that the total colony counts were less than 1,000 per plate. In less heavily contaminated environments longer periods of sampling were used.

A second method of sampling air-borne dust was to expose Petri dishes containing suitable medium to trap settling dust particles. The usual period of exposure was one hour. Colony counts were made after incubation and expressed as the number settling on 12 square inches per hour (Settling Plates).

7: BACTERIOPHAGE TYPING TESTS

Source of the typing phages

The phages were obtained from the Staphylococcal Reference Laboratory, Colindale, London. The typing set consisted of 28 phages, 18 of which were those originally described by Wilson and Atkinson (1945) and 2 isolated by the same workers at a later date (42D and 42E). Phages 53 and 54 were added by Allison. Phages 55,70, 71, 73, 75, 76, 77, 79 and 80 were isolated more recently and were obtained through the courtesy of Dr. R.E.O. Williams of Colindale Laboratory.

Propagating strains of staphylococci

These strains of Staph. aureus were also obtained from Colindale and they were maintained as previously described (page46). Nutrient broth cultures were subcultured once a week for use.

Medium for propagating and typing

Fildes digest broth containing 1.5 % shred agar (i.e. sufficient to give a rather soft plate) was used both for propagation and for typing. For the latter procedure the digest agar was underlayered with 1.5 % shred agar containing 1 % Evans peptone and 0.5 % sodium chloride. Nutrient broth was used for fluid cultures and peptone water for dilutions.

Propagation of bacteriophages

Propagation was carried out serially from batch to batch.

Freeze-dried/

Freeze-dried preparations were kept for use in case the stock cultures became contaminated or changed in character.

Petri dishes, cast to a depth of 5 mm. with digest agar, were inoculated with a broth culture of the propagating strain using a sterile glass spreader to distribute the minimum number of drops that would give confluent growth. When the culture had been absorbed into the agar the phage was spread over all save a small segment of the plate. The plates were then incubated at 37°C. overnight. The concentration of phage used was 100 times the concentration required to give confluent lysis with the propagating strain.

After incubation the control area was examined; this generally showed good growth with no evidence of spontaneous lysis. If spontaneous lysis had occurred the plates were discarded as the filtrate would be contaminated with the spontaneous phage. The control area was cut out with a sterile knife and the remaining agar frozen by holding at -60°C. for 1 hour. After freezing, the plate was allowed to thaw at room temperature and the agar gel disintegrated with bleeding of fluid. This fluid was pipetted off, centrifuged and titrated by applying 0.02 ml. drops of decimal dilutions in peptone water to a plate previously seeded with an appropriate indicator strain.

If the titre was satisfactory the filtrate was filtered through a Seitz sterilising pad and the sterile filtrate re-titrated against the propagating strain to check on loss of potency through absorption. The filtrate was also checked for identity/

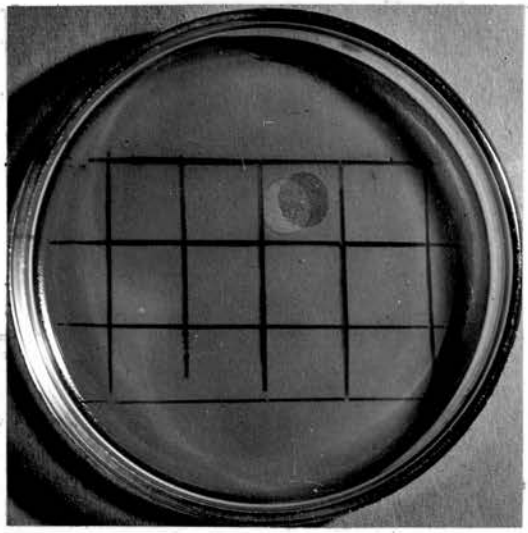
identity of the phage by spotting drops of the undiluted filtrate on plate cultures of a set of indicator strains to confirm that the lytic spectrum was unchanged.

For typing purposes the phage filtrates were used both undiluted and at their Routine Test Dilutions (R.T.D.). The R.T.D. was the highest dilution of the phage filtrate producing confluent lysis of its homologous strain. These dilutions were prepared weekly and stored in the cold when not in use.

The typing technique

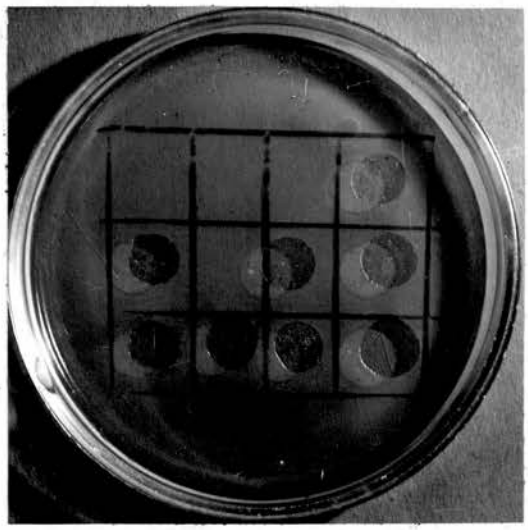
A grid of 25 squares was marked on the bottom of Petri dishes with crayon, the plates cast with peptone agar and this overlaid with digest agar to give a total depth of 3 mm. of medium. Overnight broth cultures of the strain to be typed were flooded on to the surface of the agar and excess culture fluid removed. The plates were then left on the bench to dry with the lid partly removed. The time taken to dry varied with the atmospheric conditions. When the plates were dry, drops of the typing phages, undiluted or at R.T.D., were applied with fine capillary pipettes delivering approximately 0.01 ml. Care was taken not to touch the plate when spotting on these filtrates since the test strain may be lysogenic for other test strains and carry-over may result in non-specific lysis. The drops were applied to the surface in a constant order over the marked square as shown in the figure (Fig. 9).

The plates were allowed to dry and incubated at 37°C. overnight. In the morning the typing plates were examined in good/



Bacteriophage typing plate marked with a grid for 12 phages. The strain of Staph.aureus sown on the surface has been lysed by one single phage filtrate.(e.g phage 52, therefore 'type' 52.

FIGURE 9a.



Bacteriophage typing plate similar to above but sown with a strain of Staph.aureus susceptible to many pahges.

(This type of result is common with strains susceptible to Group III phages).

FIGURE 9b.

good natural light and each square of the typing grid examined for lysis which was reported as follows:-

+++ = confluent lysis with or without resistant growth.

++ = strong lysis with 50 or more plaques.

+ = moderate lysis with 20 - 50 plaques.

The complete pattern of lysis for each strain was recorded.

EXPERIMENTAL WORK

A Study of Nasal Carriage of Staphylococcus aureus

1: POPULATION EXAMINED FOR NASAL CARRIERS

A wide range of population groups was examined for nasal carriage of Staphylococcus aureus including successive years of medical students in the 3rd, 4th and 5th year of their curriculum, patients in an urban general practice, nurses and graduate staff in several hospitals in the Edinburgh area, patients in hospital, and babies, born in hospital and outside of hospital, either at home or in nursing-homes.

Initially a single sampling of the anterior nares of these individuals was carried out and the results given are those of the first swabs from each person (Table 9).

As many persons as possible in each group were followed up by repeated sampling to investigate the length of time that Staph. aureus colonises the nose, and the nature of the strains involved. Frequent sampling was easier to carry out at regular intervals in the group of students than in the other groups because of their accessibility for periods of a year or more.

2: Recovery of Staphylococcus aureus from Individual Nostrils

A group of carriers were examined for colonisation of their right and left nostrils by using a separate swab to sample each nostril. The results shown in Table 10 show that 10 out of 76 carriers examined yielded Staph. aureus in large numbers from one /

Group	Year	Number examined	Percentage from whom Staph. aureus isolated	Isolated in large numbers (%)	Isolated in small numbers (%)
I Students	1950	47	35	26	9
	1951	150	38	29	9
	1952	183	32	24	8
	1953	175	36	26	10
	1954	163	37	27	10
	1955	153	35	25	10
	1956	85	39	28	11
	1957	76	40	24	16
	1958	76	37	29	8
	All years	1108	36	26	10
II Persons in general practice	1953	300	33	31	2
	1955	150	31	27	4
	1956	184	34	29	5
	1957	202	37	33	4
	1958	124	36	31	5
	All years	960	34	30	4
III Hospital nurses	1953	123	53	41	12
	1954	156	62	52	10
	1956	650	64	47	17
	1958	250	67	54	13
	All years	1179	63	49	14

RESULTS OF A SINGLE SAMPLING OF THE ANTERIOR NARES FOR STAPH. AUREUS IN PERSONS NOT SUFFERING FROM STAPHYLOCOCCAL DISEASE.

TABLE 9.

contd./

TABLE 9(contd.)

Group	Year	Number examined	Percentage from whom Staph. aureus isolated	Isolated in large numbers %	Isolated in small numbers %
IV Hospital patients	1953	200	48	34	14
	1954	86	42	25	17
	1956	160	42	30	12
	1958	240	43	26	17
	All years	706	44	29	15
V Babies	1957-8				
Born in hospital		295	96	88	8
Born at home		40	54	48	6

RESULTS OF A SINGLE SAMPLING OF THE ANTERIOR NARES FOR STAPH. AUREUS IN PERSONS NOT SUFFERING FROM STAPHYLOCOCCAL DISEASE.

TABLE 9 (Contd.)

Age Group		Number examined	Percentage who were persistent carriers		
			Male	Female	All persons
Babies	At birth	40	0	0	0
	At 10 days	40	40	54	48
Children	3-12 years	63	20	18	20
Adolescents	15-18 "	113	26	22	25
Young adults	19-25 "	108	33	32	34
Middle aged	30-50 "	185	28	26	28
Elderly	50 "	94	21	24	22

NASAL CARRIAGE RATE AT DIFFERENT AGES IN THE

NON-HOSPITAL POPULATION.

TABLE 17

Number of carriers examined	Number from whom Staph. aureus were isolated in large numbers		
	Right nostril only	Left nostril only	Both nostrils
76	6	4	66

RESULTS OF SEPARATELY SAMPLING NOSTRILS

OF PERSISTENT CARRIERS.

TABLE 10.

one nostril only. For this reason, in routine sampling, using only one swab, both nostrils were sampled.

3: Results of Sampling the Anterior Nares for Staphylococcus aureus.

A large number of the persons examined yielded Staph. aureus from their anterior nares.

The results of a single swabbing show that 34 per cent of those examined in general practice, 36 per cent of students, 44 per cent of patients in hospital, 63 per cent of nurses in hospital and 96 per cent of new born babies on discharge from hospital, but only 40 per cent of babies born at home, yielded Staph. aureus. The numbers of the organism isolated were small in 4 per cent of the persons in general practice, 10 per cent of the students, 15 per cent of hospital patients, 14 per cent of nurses and 8 per cent of hospital born babies (Table 9).

These results show that there was a marked difference in the number of persons living outside hospital who had Staph. aureus in their noses compared with the number inside hospital. Approximately 30 per cent of persons living outside hospital and 50 per cent of persons living inside hospital had large numbers of the organism in their nares. This contrast was also shown clearly in the case of new-born babies for twice the number born in hospital gave positive nose-swab cultures as did those born at home.

The figures in Table 9 do not show any significant change in the proportion of persons with Staph. aureus in their nares between the years 1951 and 1958.

Repeated examination of the same individuals for nasal carriage was carried out in 1,014 students who were examined for

Year	Number of persons examined	Percentage from whom Staph. aureus isolated after								Percentage from whom Staph. aureus isolated on				
		1st week	2nd week	3rd week	4th week	5th week	10th week	20th week	30th week	1 Successive occasions	2	3	4	5
1952	183	30	45	54	62	62	68	81	83	83	52	45	35	33
1953	175	33	50	53	60	61	70	76	81	81	49	37	30	27
1954	163	36	47	56	61	67	78	80	85	85	53	43	35	30
1955	153	35	45	57	67	68	72	81	83	83	48	40	35	32
1956	185	39	50	54	59	63	68	75	78	78	46	39	34	31
1957	76	40	46	50	57	62	66	70	77	77	48	42	38	33
1958	79	37	44	48	55	59	65	70	76	76	45	39	35	31
All years	1014	35	48	54	61	64	71	78	82	82	50	42	34	31

FREQUENCY OF ISOLATION OF STAPH. AUREUS FROM ANTERIOR NARES OF STUDENTS.

TABLE 11.

30 consecutive weeks during the years 1952-1958. The number from whom Staph. aureus was isolated on at least one occasion increased steadily each week, due chiefly to the number of individuals who yielded small numbers, so that about 80 per cent of those examined had been positive on at least one occasion by the 30th week, although the number positive at any one sampling was more or less constant. (Table 11). The number of persons from whom Staph. aureus was isolated on two successive occasions was much smaller than the number from whom the organisms were isolated on one occasion. The number who yielded the organism on 4 or more occasions approximates the number who had large numbers in their anterior nares (Table 9) and from whom the organism is recovered regularly over a longer period (Table 12). Thus persons from whom Staph. aureus was isolated on the great majority of occasions om which they were sampled have been called persistent carriers, and the proportion of these among the students examined in successive years is shown in (Table 12). Staph. aureus was isolated less regularly from others who have been called intermittent carriers ; and the remaining persons from whom Staph. aureus was isolated have been called temporary carriers since the organism was isolated on only a single or few occasions during the period of observation.

Repeated examinations were also made in groups of nurses, although these could not be as regular nor as extended as in the student group. The results in Table 13 show the same features as described for the students. The number from whom Staph. aureus was isolated on first swabbing was about 60 per cent ; by the fifth /

YEAR	NUMBER OF PERSONS EXAMINED	PERCENTAGE FROM WHOM STAPH. AUREUS ISOLATED ON AT LEAST ONE OCCASION	PERCENTAGE WHO WERE		
			PERSISTENT CARRIERS	INTERMITTENT CARRIERS	TEMPORARILY CONTAMINATED
1952	183	83	25	14	44
1953	175	81	23	13	45
1954	163	85	27	18	40
1955	153	83	26	15	42
1956	185	78	24	14	44
1957	76	77	23	12	42
1958	79	76	21	15	40
ALL YEARS	1014	82	25	14	44

Relative number of different types of nasal carrier of Staph.Aureus among students.

TABLE 12.

Year	Number of persons examined	Percentage from whom Staph. aureus isolated after					Percentage from whom Staph. aureus isolated on				
		1st	2nd	3rd	4th	5th	1	2	3	4	5
		Examinations					Successive occasions				
1953	108	62	70	73	79	88	88	65	49	47	45
1954	86	58	65	70	79	89	89	60	48	43	48
1956	580	63	71	78	81	87	87	61	53	49	45
1958	100	57	62	68	74	78	78	58	50	48	44
All years	874	62	70	73	77	84	84	61	51	48	44

FREQUENCY OF ISOLATION OF STAPH. AUREUS

FROM ANTERIOR NARES OF NURSES.

TABLE 13.

fifth swabbing over 80 per cent had yielded the organism on at least one occasion. The number of nurses yielding Staph. aureus on 3 or more successive occasions was less than 50 per cent, again corresponding to the number from whom large numbers of staphylococci were isolated (Table 9).

The results of repeated examination of patients in general practice were similar to those of the previous groups, although the increase in the number of persons who yielded Staph. aureus at each examination was less. This corresponded to the smaller number of persons in this group who had small numbers of organisms in their nares (Table 14).

4: The persistence of nasal carriage

A number of students were examined for nasal carriage of Staph. aureus at fairly regular intervals, over a longer period of time, to investigate the length of time that the nares remained colonised with the organism. The majority who were colonised at the start of the investigation persistently yielded Staph. aureus from their anterior nares for over 3 years, and in one instance at least for over 5 years. In most cases the strain isolated was identical in bacteriophage pattern and antibiotic susceptibility each time it was isolated (Table 15).

Among 358 students observed during 1951 and 1952, 26 persistent and intermittent carriers had become non-carriers at the end of 12 months, while 24 non-carriers had become persistent or intermittent carriers (Table 16). The number who ceased to be carriers was therefore 7.3 per cent of all the students examined in each 12 months and may be taken as a measure of the persistence

of/

YEAR	NUMBER OF PERSONS EXAMINED	Percentage from whom Staph. Aureus isolated after				Percentage from whom Staph. Aureus isolated on			
		1st	2nd	3rd	4th	1	2	3	4
		Examination				Successive Occasions			
1953	120	38	41	44	-	44	35	32	-
1955	80	34	36	42	46	46	34	30	27
1956	86	33	37	44	48	48	33	31	29
1957	68	33	38	42	47	47	36	34	33
All years	354	35	38	43	47	48	35	32	30

Frequency of isolation of Staph.Aureus from anterior nares of patients in general practice (persons not in hospital).

TABLE 14.

Person examined		ISOLATION OF STAPH. AUREUS AND IDENTIFYING CHARACTERS								
		Initial examination	After 1 month	2 months	6 months	12 months	24 months	30 months	36 months	48 months
PHI	I*	+++	+++	+++	++	+++	+++	+++	++	+++
	P*	PS	PS	PS	PS	PS	PS	PS	PS	PS
	T*	52/52A	52/52A	52/52A	52/52A	52/52A	52/52A	52/52A	52/52A	52/52A
DOU	I	-	-	-	-	-	-	-	-	-
	P	.	.	.	.	.	.	.	.	.
	T	.	.	.	.	.	.	.	.	.
LAM	I	+++	+++	+++	+++	+++	+++	+++	.	.
	P	PR	PR	PR	PR	PR	PR	PR	.	.
	T	6/7/47/47A/54	6/7/47/47A/54	6/7/47/54	6/7/47/54	6/7/47/47A/54	6/7/47/47A/54	6/7/47/54	.	.
GO	I	++++	++++	+++	+++	+++	++++	++++	++++	++++
	P	PR	PR	PR	PR	PR	PR	PR	PR	PR
	T	52	52	52	52	52	NT	NT	NT	NT

RESULTS OF SAMPLING THE ANTERIOR NARES OF A NUMBER OF INDIVIDUALS OVER A LONG PERIOD

* I = Isolation
* P = Penicillin susceptibility
* T = Phage type

TABLE 15.

contd./

NUMBER OF STUDENTS EXAMINED	PERCENTAGE WHO CHANGED FROM			
	NON-CARRIER TO INTERMITTENT CARRIER	INTERMITTENT TO PERSISTENT CARRIER	INTERMITTENT TO NON-CARRIER	PERSISTENT TO NON-CARRIER
358	2.6	4.4	1.7	5.6
	7.0		7.3	

Number of student carriers who changed their nasal carriage status during 12 months observation.

TABLE 16.

of nasal carriage ; thus the average length of time that any one person may be expected to carry Staph. aureus equals $\frac{100}{7.3}$ = 14 years. Persistence for such a long time is likely to ensure a more or less constant carriage rate till middle age, and this seems to be the case (Table 17). p 94

5: Results of typing carrier strains of Staph. aureus with Bacteriophages

All of the strains isolated from carriers were tested for their susceptibility to the stock set of staphylococcal bacteriophages. The patterns of lysis produced have been grouped according to the classification proposed by Williams, Rippon and Dowsett,(1953).

About 80 per cent of all the strains of Staph. aureus tested were susceptible to lysis with one or more of the phages, either at routine test dilutions (R.T.D.) or with the undiluted filtrates (Table 18).

Of the strains isolated from students 26 per cent had patterns belonging to Group III ; 27 per cent were of Group I and 14 per cent of Group II. The proportion of Group I and III strains isolated in general practice were similar but there was a larger number of Group II strains, and fewer that did not type with the phages. Among successive years of medical students and persons in the general population the proportion of strains of each group remained fairly constant and correspond approximately to those obtained elsewhere by other observers. (Table 18).

The proportions of Group I and Group III patterns among strains isolated from nurses in hospital were very different ; more than half were of Group III, and only a small minority were of Group II.

The/

Group of Persons Examined	Number of Strains Examined	Percentage of Strains of Staph. aureus with Phage Susceptibility Patterns of				
		Group I	Group 11	Group 111	Unclassified	Not Typable
I Students						
1951	150	26	15	24	9	26
1952	176	26	15	19	5	35
1953	134	22	14	26	8	29
1954	146	27	15	30	6	22
1955	157	29	16	26	5	24
1956	88	28	12	30	8	22
1957	96	28	14	32	4	22
1958	72	34	12	28	4	22
All YEARS	1019	27	14	26	7	26
II						
1953	121	24	30	21	6	10
1955	117	22	28	26	10	14
1956	131	32	24	26	5	13
1957	121	29	22	29	8	12
1958	58	32	20	25	7	16
ALL YEARS	548	27	25	25	8	15

BACTERIOPHAGE TYPING RESULTS OF ALL STRAINS ISOLATED FROM PERSONS EXAMINED

TABLE 18.

Group of Persons Examined	Number Of Strains Examined	Percentage of Strains of Staph. aureus with phage susceptibility patterns of				
		Group I	Group II	Group III	Unclass-ified	Not Typable
III						
1953	80	15	4	70	3	8
1954	135	12	8	69	0	10
1956	491	24	6	49	1	20
1958	121	30	7	42	6	15
All Years	827	22	6	54	2	16

TABLE 18 (Contd.)

YEAR	Number of Strains Examined	Percentage with Bacteriophage Lytic Patterns belonging to Group I	Group II	Group III	Unclass-fied	Not Typable
IV						
1950	19	30	17	35	9	9
1953	101	21	20	28	14	17
1954	31	28	19	30	6	17
1956	63	31	14	33	9	13
1958	98	27	19	29	6	19
All Years	312	26	18	30	9	17
V						
Babies Born in Hospital S	195	44	4	45	2	5
Babies Born in Hospital E	100	16	5	74	0	4

TABLE 18(Contd).

The proportion of Group patterns of each group among strains isolated from hospital patients was similar to those isolated from patients in general practice.

An examination of the figures obtained for successive years from 1951 showed that there was a decrease in the porportion of Group II strains from general practice, and a corresponding increase in Group I strains. A more marked change in the distribution of the patterns was seen among strains isolated from nurses in hospital (Table 18) as twice the number of Group I strains were recovered in 1958 than in 1953, and this corresponded with a fall in the number of strains with Group III patterns. Group II strains remained very much in the minority. There was little change in the distribution of strains of different patterns among strains isolated from patients in hospital over the same period.

6: Occurrence of Individual Patterns of Bacteriophage lysis

Tables 19-22 show the frequency of individual patterns of phage lysis among strains examined from the different population groups.

A very large number of distinct patterns was obtained from strains isolated from the students and persons in general practice, and the individual patterns were thus not repeatedly recorded. Generally speaking the same patterns were found to occur among these two groups of individuals. The total number of distinct patterns was more than 200, many of them occurring only once. The most frequently recurring patterns were in bacteriophage group I and II.

A much smaller number of distinct patterns was found among /

among strains isolated from hospital nurses. Moreover the majority of strains in any one hospital belonged to a small number of different patterns. Thus in Hospital 'R' (Table 21) 69 per cent of strains belonged to 7 patterns, and 45 per cent to 2 patterns, and in hospital 'WE' 59 per cent of the carrier strains were distributed among 7 patterns. (Table 22).

TABLE 19.

Bacteriophage PATTERN	Number of Strains	Percentage of Typable Strains	Number of Strains in Group	Group
29	34	5		
29/52	26	3		
29/52/52A	31	4	276	I
52/52/A	41	6		
52	16	2		
52A	12	2		
Others	116	15		
3A	15	2		
3A/3B	20	3		
3A/3B/3C	25	3		
3A/3B/3C/51/55	34	5		
3B/3C	16	2		
3B	5	1	146	II
3C	4	1		
3B/3C/51	8	1		
3C/51	7	1		
Others	14	2		
6/7/47/54	26	4		
7/47/53/54	28	4		
47/53/77	16	2	266	III
Others	196	26		
Miscellaneous	65	9	65	

FREQUENCY OF OCCURRENCE OF INDIVIDUAL BACTERIOPHAGE PATTERNS IN 753 STRAINS ISOLATED FROM STUDENTS.

BACTERIOPHAGE PATTERN	NUMBER OF STRAINS	PERCENTAGE OF TYPABLE STRAINS	NUMBER OF STRAINS IN GROUP	BACTERIOPHAGE GROUP
29	20	4		
29/52	14	3		
29/52/52A	16	3		
52/52A/80	6	1	150	I
52A	18	4		
OTHERS	76	16		
3A	6	1		
3A/3B	9	2		
3A/3B/3C	17	4		
3A/3B/3C/51	18	4		
3A/3B/3C/51/55	22	5	140	II
3B/3C	13	3		
3B/3C/51	11	2		
51	5	1		
OTHERS	39	8		
79	17	4		
6/7/47/53/54	14	3		
OTHERS	108	23	139	III
MISCELLANEOUS	40	8	40	

Frequency of occurrence of individual bacteriophage patterns in 469 strains isolated from persons in general practice.

TABLE 20.

BACTERIOPHAGE PATTERN	NUMBER OF STRAINS	PERCENTAGE OF TYABLE STRAINS	PERCENTAGE OF PATTERNS IN GROUP
52/52A	14	5	
42B/52/52A/80	18	7	I 20
52/79/80	7	3	
OTHERS	14	5	
3B/3C	5	2	II 7
OTHERS	14	5	
54/76/77	39	15	
53	24	9	
53/75	20	7	III 66
6/7/44/47/70/73/75	36	14	
OTHERS	57	21	
MISCELLANEOUS	19	7	UNCLASSIFIED 7

Frequency of occurrence of individual bacteriophage lytic patterns among 267 carrier strains of Staph.Aureus isolated in hospital R

TABLE 21.

BACTERIOPHAGE PATTERN	NUMBER OF STRAINS	PERCENTAGE OF TYPABLE STRAINS	PERCENTAGE OF PATTERNS IN GROUPS
52/52A/80	96	23	
42B/73/80	19	5	I 32
OTHERS	14	4	
3A	24	5	
3A/3C/51	3	1	II
OTHERS	5	1	
54/75/76/77	94	22	
7/54	26	5	
53/77	26	5	III 60
47/54/73	17	4	
OTHERS	94	22	
MISCELLANEOUS	8	1	1

Frequency of occurrence of individual bacteriophage lytic patterns among 426 carrier strains of Staph.Aureus isolated in hospital 'WE'

TABLE 22.

7: Nasal carriage of Staph. aureus at different ages

The incidence of nasal carriers at different ages among 513 of the nasal carriers who were examined in the non-hospital community are given in Table 23.

At birth the new-born had no staphylococci on the skin of their nasal orifices but they were rapidly colonised and at 10 days old about a half of them yielded a profuse growth of Staph. aureus. The proportion of nasal carriers among children up to 12 years of age was relatively small and therefore many of the babies who were colonised at an early age must have lost their nasal staphylococcus. As age increased so did the proportion of nasal carriers and more than one third of young adults were colonised in the nose. The proportion of middle-aged and elderly persons who were carriers was smaller and may indicate the development of an immunity.

Repeated examination of the same individuals for Staph. aureus in the anterior nares has confirmed that the presence of the organism is sporadic in some and more constant in others.

Bacteriophage typing of the strains isolated from these individuals enables a more clear cut classification to be made so that it is clear that many are merely temporary hosts of Staph. aureus. Therefore the mere isolation of Staph. aureus from the anterior nares is not sufficient evidence of the carrier state. A carrier is therefore better defined as an individual who carries the same strain of staphylococcus persistently in the nose.

Temporarily contaminated persons were those individuals from whom Staph. aureus was isolated on one or a few separate occasions/

AGE GROUP	NUMBER EXAMINED	PERCENTAGE PERSISTENT NASAL CARRIERS	PERCENTAGE CARRIERS M	F
At birth	40	0	0	0
10 days old	40	48	40	54
3 - 12 yrs.	63	20	22	18
15 - 18	113	25	26	22
19 - 25	108	36	32	34
30 - 50	85	28	29	26
50	64	22	21	24

Nasal carriage rate of Staph. Aureus at different ages in the non-hospital community.

TABLE 23.

7: Nasal carriage of Staph. aureus at different ages.

The incidence of nasal carriers at different ages among 513 of the nasal carriers who were examined in the non-hospital community is given in Table,23.

At birth the new-born had no staphylococci on the skin of their nasal orifices but they were rapidly colonised and at 10 days after birth about one half of them yielded a profuse growth of Staph.aureus. The proportion of nasal carriers among children up to 12 years of age was relatively small and therefore many of the babies who were colonised at an early age must lose their nasal staphylococci as they grow older. In adolescence the proportion of carriers increased until more than one-third of young adults were found to be colonised with Staph.aureus. The proportion of middle-aged and elderly persons who were carriers was smaller and may indicate the development of resistance to colonisation. (Table 17.)

Repeated examination of the same individuals for Staph. aureus in the anterior nares has confirmed that the presence of the organism is sporadic in some and more constant in others.

Bacteriophage typing of the strains isolated from these individuals enables a more clear cut classification to be made so that it is clear that many are merely temporary hosts of Staph. aureus. Therefore the mere isolation of Staph. aureus from the anterior nares is not sufficient evidence of the carrier state. A carrier is therefore better defined as an individual who carries the same strain of staphylococcus persistently in the nose.

Temporarily contaminated persons were those individuals from whom Staph. aureus was isolated on one or a few separate occasions during the period of observations, the number of isolations rarely exceeding 10 % of the examinations in any one individual. The staphylococci isolated on different occasions from the same individual were usually of different phage type and sometimes of different susceptibility to antibacterial substances. In these persons the staphylococci were assumed to be chance contaminants drawn into the nares with the dust of the air and filtered off as has been described in the case of streptococci by De Waal (De Waal, 1940). These staphylo-:cocci did not survive long in the nose and did not appear to multiply to any extent.

In the majority (over 80 %) of these contaminated individuals/

individuals a few colonies only were isolated at each swabbing. A few (no more than 5 %) had a heavier growth of Staph. aureus on a single occasion and this probably indicated multiplication for a limited period, but in none did there appear to be true parasitism by the staphylococcus since repeated samplings did not yield the same organism.

Intermittent carriers were those individuals examined in whom periods of a few weeks carriage alternated with similar periods when no Staph. aureus were isolated. However, the staphylococcus isolated was always of the same phage type in any one individual and this seems to distinguish them from occasional or contaminated persons re-infected with different strains.

These intermittent carriers must be regarded as true hosts of Staph. aureus as it would appear that this organism living and multiplying in the skin of the nares, is only present on the surface in large enough numbers for isolation at certain periods. From the results there is some evidence that an intermittent carrier may become progressively more regular in yielding positive cultures of Staph. aureus so becoming a persistent carrier. Within the group of intermittent carriers as a whole there was a great deal of variation both in the frequency of isolation of the staphylococcus and in the number of colonies obtained in each culture on successive examinations. Some individuals yielded a relatively poor growth on only half-a-dozen occasions throughout the year whilst others had a heavy growth for many weeks on end.

Some persons with cultures negative for Staph. aureus over a long period of time and who were regarded as non-carriers do tend to become intermittent carriers. Thus it may be that the intermittent carrier represents a phase or step in the development or colonisation of a persistent carrier.

Persistent carriers were individuals from whom Staph.aureus of the same phage type was isolated on more than 90 % of the occasions on which they were examined. These persons may carry a staphylococcus of the same phage type for several years (Table 15). The great majority of these carriers yield large numbers of colonies on cultures of their swabs on each occasion of sampling and it must be concluded that the organism was well established in this site, multiplying at a great rate and cover-:ing the surface of the anterior nares in such numbers that it was readily sampled.

During the period of observation a few persistent carriers ceased to yield staphylococci from their nares and seemed to become non-carriers. In a few this was a permanent change. Although persistent carriers were observed to develop from intermittent carriers there were no instances of their develop-:ment from previously consistent non-carriers. The inter-:mittent carrier state may therefore be an essential transition-:al phase in the establishment of the carrier state.

The total number of individuals from whom Staph. aureus may be isolated increases steadily with the number of examin-:ations made so that in any one group examined regularly over

a/

a period of a few months more than 80 % will have yielded Staph. aureus; as has been explained this included persons contaminated with the organism from the environment and is not related to the number of persons actually colonised with the organism.

From Table 11 it can be seen that the number of persons yielding Staph. aureus on two successive occasions was very much lower than for a single isolation, while the number from whom Staph. aureus was isolated on 5 or more successive occasions approximates to the number of intermittent and persistent carriers.

Thus a useful criterion for distinguishing carriers in community would be the isolation of a staphylococcus of the same phage type in large numbers on at least 3 successive occasions.

8: Results of Testing the Susceptibility of Staphylococcus Aureus to Antibiotics

The results of testing susceptibility to penicillin and the antibiotics other than penicillin are considered separately.

(a) Susceptibility to penicillin

The majority of the strains of Staph. aureus tested by the disc diffusion technique, and giving a zone of inhibition of growth with a lytic margin, had a minimum inhibitory concentration approximately the same as the standard staphylococcus (0.02 μg/ml), and in all cases less than 0.1 μg per ml.

Strains with zones of inhibition of growth showing no lysis required more than 0.1 μg per ml. for inhibition, and without exception produced penicillinase. The diameter of the zone of inhibition of growth in the disc diffusion test, and the minimum inhibitory concentration determined by the serial tube dilution technique varied with the size of the inoculum used. Under the present conditions of test the majority of these penicillinase producing strains required between 1 and 100 μg. per ml. for inhibition of growth.

Thus Staphylococcus aureus was divided into two sharply defined groups on the basis of susceptibility to penicillin in the disc diffusion test. Those strains showing a zone of inhibition of growth with marginal lysis were regarded as susceptible ; those without a zone of inhibition, or a zone showing no marginal lysis, were regarded as resistant.

The majority of strains isolated from persons outside hospital/

hospital were susceptible to penicillin. The proportion of susceptible strains was greatest among patients in general practice (Table 24). However the number of penicillin resistant strains isolated increased each year, both among the patients in general practice and the students (Tables 25,26).

The strains of Staph. aureus isolated from hospital nurses were nearly all resistant to penicillin, as were those isolated from the babies born in hospital. Only half of the strains isolated from babies born outside hospital were resistant to penicillin.

The majority of strains of Staph. aureus isolated from carriers among patients in hospital were susceptible to penicillin.

The difference between the proportion of penicillin-resistant strains isolated from the hospital and non-hospital populations is even greater when we consider the susceptibility of the strains isolated from persistent carriers only. Over 80 per cent of the strains isolated from students and patients in general hospital were penicillin-susceptible, whereas less than 10 per cent isolated from nurses were in this category. (Table 28).

The increase in the proportion of penicillin-resistant strains isolated from persistent carriers in the non-hospital population is shown in Tables 25-27. In 1958 about a third of the strains were resistant to penicillin compared with less than a fifth in 1953. A similar change was observed in the strains isolated from hospital nurses who were persistent carriers, only 5 per cent being penicillin-susceptible in 1958 compared /

SOURCE OF STRAINS	NUMBER OF STRAINS EXAMINED	PERCENTAGE OF STRAINS SUSCEPTIBLE TO PENICILLIN 0.1µg/ml.	PERCENTAGE OF STRAINS RESISTANT TO PENICILLIN 0.1µg/ml.
STUDENTS	1019	64	36
GENERAL PRACTICE	548	76	24
HOSPITAL NURSES	827	13	87
HOSPITAL PATIENTS	312	69	31
HOSPITAL BABIES	295	7	93
OTHER BABIES	40	50	50

Penicillin susceptibility of all carrier strains of Staphylococcus Aureus.

TABLE 24.

YEAR	NUMBER OF STRAINS EXAMINED	PERCENTAGE OF STRAINS SUSCEPTIBLE TO PENICILLIN	PERCENTAGE OF STRAINS RESISTANT TO PENICILLIN
1949	43	88	12
1951	71	86	14
1952	61	95	5
1953	71	78	22
1954	78	82	18
1955	89	74	26
1956	37	72	28
1957	33	70	30
1958	31	64	36
ALL YEARS	514	80	20

Penicillin susceptibility of strains of Staph. Aureus isolated from persistent carriers among students.

TABLE 25.

YEAR	NUMBER OF STRAINS EXAMINED	PERCENTAGE OF STRAINS SUSCEPTIBLE TO PENICILLIN	PERCENTAGE OF STRAINS RESISTANT TO PENICILLIN
1955	44	90	10
1956	38	81	19
1957	31	71	29
1958	27	67	33
ALL YEARS	140	83	17

Penicillin susceptibility of strains of Staph.Aureus isolated from persistent carriers among patients in general practice.

TABLE 26.

YEAR	NUMBER OF STRAINS EXAMINED	PERCENTAGE OF STRAINS SUSCEPTIBLE TO PENICILLIN	PERCENTAGE OF STRAINS RESISTANT TO PENICILLIN
1953	49	14	86
1954	56	16	84
1956	380	8	92
1958	75	5	95
ALL YEARS	560	9	91

Penicillin susceptibility of strains of Staph.Aureus isolated from persistent carriers among hospital nurses.

TABLE 27.

SOURCE OF STRAINS	NUMBER OF STRAINS EXAMINED	PERCENTAGE OF STRAINS SUSCEPTIBLE TO PENICILLIN	PERCENTAGE OF STRAINS RESISTANT TO PENICILLIN
I STUDENTS	514	80	20
II GENERAL PRACTICE	140	83	17
III NURSES	560	9	91

Penicillin susceptibility of strains of Staph. Aureus isolated from persistent carriers.

TABLE 28.

compared with 14 per cent in 1953.

2: (b) Susceptibility to antibiotics other than penicillin.

In the majority of instances the cultures tested were either as susceptible as the standard organism or were many times as resistant, giving little or no zone of inhibition of growth around the routine antibiotic discs. In a small number the zone of inhibition was a few millimetres smaller in diameter than with the standard organism or there was a ring of discrete colonies (resistant variants) within the zone of inhibition of the majority of the bacterial population. (Figs 7, 8)

The following concentrations, required for inhibition of growth, and the equivalent zone diameters with routine discs, were taken as indicating resistance to the antibiotics concerned.

ANTIBIOTIC	Conc. for Inhibition (M.I.C.) µg/ml.	Zone diameter Using standard Test Disc mm.
Streptomycin	5	13
Chloramphenicol	5	20
Chlortetracycline	5	15
Oxytetracycline	5	18
Etrythromycin	2.5	18

The susceptibility of all the strains isolated from the various population groups is shown in Table 30 .

The outstanding observation was that none of the strains which were penicillin-susceptible and producing no penicillinase, were resistant to other antibiotics. Thus all of the strains resistant/

SOURCE OF STRAINS	NUMBER EXAMINED	PERCENTAGE OF STRAINS RESISTANT TO				
		PENICILLIN	STREPTOMYCIN	CHLORAM PHENICOL	TETRACYCLINE	
STUDENTS	1019	36	5	1	1	0
GENERAL PRACTICE	548	24	9	4	2	0
HOSPITAL NURSES	827	87	54	9	24	1
HOSPITAL PATIENTS	312	31	15	3	1	0
BABIES	295	93	58	12	30	6

Relative numbers of strains of Staph.Aureus resistant to the different antibiotics.

TABLE 29.

resistant to streptomycin, chloramphenicol, the tetracyclines or erythromycin were penicillinase producers. (Table 30).

Again the results differ from the strains of Staph. aureus isolated from the non-hospital community and hospital nurses.

3: Strains from the non-hospital community, Students and General Practice patients.

Only 37 strains resistant to antibiotics other than penicillin were isolated during seven years observation, and 28 of these were resistant only to penicillin and streptomycin ; 6 were resistant to tetracyclines in addition, and 3 to chloramphenicol, 2 of these 3 also being resistant to tetracyclines. No strains resistant to erythromycin were observed. (Table 30).

The majority of the resistant strains had bacteriophage lytic patterns belonging to Group III. No strain with a Group II pattern, resistant to chloramphenicol or tetracyclines was isolated (Tables 32-36 and Fig. 10)

4: Hospital community

A large proportion of the strains isolated from carriers in the hospital community exhibited resistance to multiple antibiotic although the patterns of susceptibility were relatively limited.

The majority of the resistant strains were of phage group III. Strains of identical bacteriophage pattern were not always of the same antibiotic susceptibility (see another section) .

The erythromycin-resistant strains were isolated in the/

the main from one hospital, 'E', and all were isolated after the introduction of the antibiotic.

The proportion of strains resistant to streptomycin, chloramphenicol and the tetracyclines isolated from hospital born babies was similar to that of the nurses strains. A larger number of erythromycin-resistant strains were isolated from the babies than from any other group and these strains were all isolated in Hospital 'E'(Table 30).

5: Susceptibility to antibiotics not in general use.

A number of strains of Staph. aureus isolated from nasal carriers were tested for their susceptibility to a number of less commonly used antibacterial agents. (Table 31).

The minimum inhibitory concentration regarded as indicating resistance was chosen in an arbitrary manner as no reliable guides to in vivo inhibitory concentrations were available.

6: Antibiotic Susceptibility Patterns

The reaction of each strain of Staph. aureus to the different antibiotics tested gave a pattern of susceptibility. The strains were classified as either resistant or susceptible to each of the antibiotics penicillin, streptomycin, chloramphenicol, tetracycline and erythromycin, and therefore the pattern of a strain susceptible to all of these antibiotics was written PS:SS:CS:TS:ES:, and of one resistant to them all, PR:SR:CR:TR:ER. Theoretically 64 different patterns of this type were possible but in actual practice the number of patterns observed was relatively small/

small, especially as all penicillin-susceptible strains were susceptible to other antibiotics, and almost all of the strains resistant to chloramphenicol, the tetracyclines and erythromycin were both penicillin and streptomycin resistant. Therefore the commonly occurring susceptibility patterns were as follows:

PS. SS. CS. TS. ES.

PR. SS. CS. TS. ES.

PR. SR. CS. TS. ES.

PR. SR. CS. TR. ES.

PR. SR. CR. TR. ES.

PR. SR. CR. TR. ER.

and only a handful of strains in this investigation were found to have other susceptibility patterns.

The frequency of these antibiotic-susceptibility patterns among the strains examined is shown in Table 30.

Only a small number of strains isolated from the non-hospital community were resistant to penicillin, streptomycin and other antibiotics; none were resistant to erythromycin. Almost a quarter of the strains isolated from nurses in hospital and hospital born babies were resistant to the tetracyclines, and/or chloramphenicol, and two-dozen strains were resistant to erythromycin.

7: Bacteriophage pattern and antibiotic susceptibility

Fifty-three per cent of penicillin-resistant strains isolated from students belonged to Phage Group III while only

SOURCE OF STRAINS	NUMBER OF STRAINS EXAMINED	NUMBER OF STRAINS WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN				
		PS and S to other antibiotics	PR:SS:CS:AS:TS:ES	PR:SR:AS:TS:CS:ES	PR:SR:CR ES or TR AR ER	PS and R to other antibiotics
STUDENTS	514	415	75	21	3	0	0
GENERAL PRACTICE	140	116	11	7	6	0	0
HOSPITAL NURSES	560	50	206	171	125	8	0
HOSPITAL PATIENTS	312	219	46	37	10	0	0
HOSPITAL BORN BABIES	295	21	103	60	95	16	0
BABIES BORN OUTSIDE HOSPITAL	40	20	14	6	0	0	0

Susceptibility to penicillin and other antibiotics of strains of Staph.Aureus isolated from nasal carriers.

TABLE 30.

ANTIBIOTIC OR ANTIBACTERIAL AGENT	NUMBER OF STRAINS EXAMINED			PERCENTAGE OF STRAINS	
				PENICILLIN-SENSITIVE	PENICILLIN-RESISTANT
POLYMYXIN	200	Percentage of Strains	Polymyxin Sensitive	69	5
			Polymyxin Resistant	11	15
NEOMYCIN	200	Percentage of Strains	Neomycin Sensitive	75	21
			Neomycin Resistant	0	4
BACITRACIN	200	Percentage of Strains	Bacitracin Sensitive	80	17
			Bacitracin Resistant	0	3
CHLORHEXIDENE	400	Percentage of Strains	Chlorhexidene Sensitive	73	17
			Chlorhexidene Resistant	7	3
FURADANTIN	200	Percentage of Strains	Furadantin Sensitive	79	16
			Furadantin Resistant	1	4

Susceptibility of selected penicillin-susceptible and penicillin-resistant strains of Staph.Aureus to some lesser used antibiotics and antibacterial agents.

TABLE 31.

6 per cent were in Group II, although strains of these groups occurred in the ratio of 3:2, (Table 32.) The preponderance of phage Group III strains, resistant to penicillin, was not so obvious among the strains of Staph. aureus from general practice, (Table 33,)but was among the strains from nurses where 61 per cent of penicillin-resistant strains were of Group III, although only 54 per cent of all strains were of this group, (Tables 32-34.)

Among the strains isolated from students, none of the Group II strains examined were resistant to antibiotics other than penicillin, and only one Group II strain isolated from persons in general practice was resistant to other antibiotics. All of the student strains resistant to streptomycin and the other antibiotics belonged to Group I or III, but most of the similar strains isolated from general practice patients were of Group I.

of the strains isolated from nurses, the majority resistant to streptomycin and penicillin but susceptible to the other antibiotics were of Group III, and the majority of those strains resistant to tetracyclines and chloramphenicol were of Group I. Only 2 strains belonging to Group I were resistant to antibiotics other than penicillin.

The strains isolated from babies born in hospital corresponded in these respects to the hospital nurses' strains and those isolated from hospital patient carriers corresponded to those isolated from persons in general practice though there was a higher proportion of antibiotic-resistant strains belonging /

belonging to bacteriophage typing groups I and III (Tables 35 and 36).

BACTERIOPHAGE GROUP	NUMBER OF STRAINS SUSCEPTIBLE TO PENICILLIN	RESISTANT TO PENICILLIN	WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN PR:SS:CS: TS:ES	PR:SR:CS:TS:ES	PR:SR: CS:TR: ES	PR:SS: CR:TR: ER
I	134	27	17	9	1	0
II	96	6	6	0	0	0
III	104	52	38	12	2	0
UNCLASSIFIED	32	4	4	0	0	0
NOT TYPABLE	46	10	10	0	0	0

Antibiotic susceptibility in relation to bacteriophage group of 511 carrier strains of Staph.Aureus isolated from students.

TABLE 32.

BACTERIOPHAGE GROUP	NUMBER OF STRAINS SUSCEPTIBLE TO PENICILLIN	RESISTANT TO PENICILLIN	WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN PR:SS:CS: TS:ES	PR:SR:CS: TS:ES	PR:SR:TR/ CR:ES:TS: CS	PR:SR:TR: CR:ER
I	28	10	4	2	4	0
II	43	2	1	1	0	0
III	16	10	5	3	0	0
IUNCLASSIFIED	7	1	0	1	0	0
NOT TYPABLE	12	1	1	0	0	0

Antibiotic susceptibility in relation to bacteriophage group of 140 carrier strains of Staph.Aureus isolated from persons in general practice.

TABLE 33.

BACTERIOPHAGE GROUP	NUMBER OF STRAINS SUSCEPTIBLE TO PENICILLIN	RESISTANT TO PENICILLIN	WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN PR:SS:CS: TS:ES	PR:SR:CS: TS:ES	PR:SR:TR/ TS:CR/CS: ES	PR:SR:CR: TR:ER
I	13	118	33	14	70	1
II	20	32	30	0	2	0
III	8	296	110	131	48	7
UNCLASSIFIED	4	8	5	2	1	0
NOT TYPABLE	5	56	28	24	4	0

Antibiotic susceptibility in relation to bacteriophage group of 560 carrier strains of Staph.Aureus isolated from nurses in hospital.

TABLE 34.

BACTERIOPHAGE GROUP	NUMBER OF STRAINS SUSCEPTIBLE TO PENICILLIN	RESISTANT TO PENICILLIN	WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN PR:SS:CS:TS: ES	PR:SR:CS: TS:ES	PR:SR:TR/ TS:CR/CS; ES	PR:SR: CR:TR:ER
I	57	25	7	13	5	0
II	42	14	14	0	0	0
III	60	34	11	20	3	0
UNCLASSIFIED	22	7	6	0	1	0
NOT TYPABLE	38	13	10	2	1	0

Antibiotic susceptibility in relation to bacteriophage group of 312 carrier strains of Staph.Aureus isolated from hospital patients.

TABLE 35.

BACTERIOPHAGE GROUP	NUMBER OF STRAINS					
	PS	PR	WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN PR:SS:CS TS:ES	PR:SR:CS TS:ES	PR:SR:CS/CR TS/TR:ES	PR:SR:CR TR:ER
I	6	96	21	11	64	0
II	6	7	6	1	0	0
III	6	157	50	32	57	18
UNCLASSIFIED	0	4	3	1	0	0
NOT TYPABLE	3	11	6	2	3	0

Antibiotic susceptibility in relation to bacteriophage group of 295 strains of Staph.Aureus isolated from hospital born babies.

TABLE 36

ANTIBIOTIC SUSCEPTIBILITY PATTERN

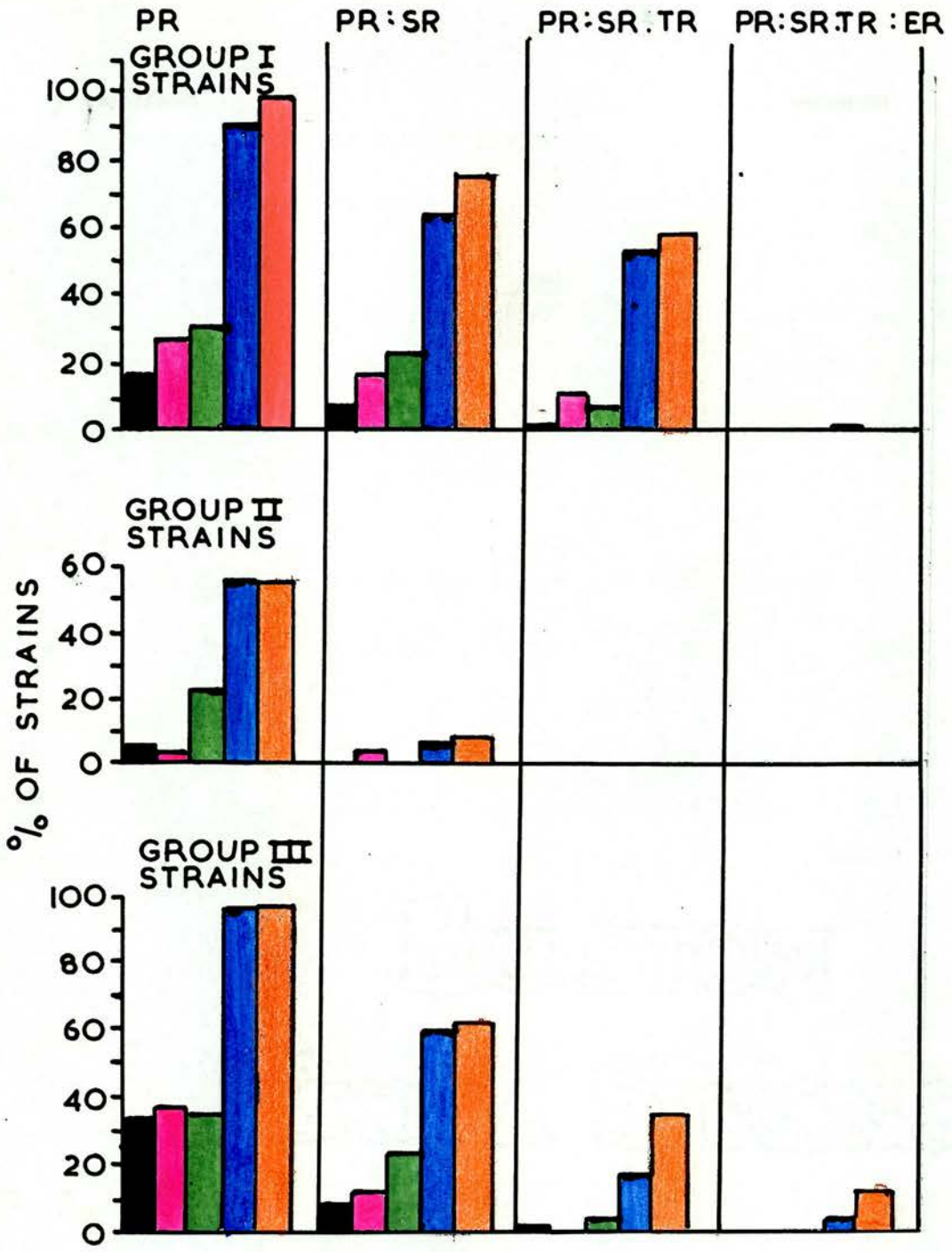


FIGURE 10.

9. Comparison of Strains of Staph. aureus isolated from Carriers and temporarily contaminated persons.

The distribution of strains belonging to the various bacteriophage groups was different in carriers and temporarily contaminated persons. These differences were most marked in the student group, though the same features were observed among the general practice and hospital nurses strains (Table 37). In addition the proportion of penicillin-resistant strains isolated from temporarily contamonated persons was considerably higher than from carriers.

The relationship between phage group and penicillin-resistance is shown in Table 38. The highest proportion of penicillin-resistant strains isolated from the carriers were of Group III patterns ; this was also true of the strains isolated from temporarily contaminated persons though their proportion was twice as great. A large proportion of the strains of unclassified patterns, and those unsusceptible to the phages were also penicillin resistant.

SOURCE OF STRAINS	NUMBER OF STRAINS	PERCENTAGE WITH PHAGE PATTERN OF GROUP					PERCENTAGE PENICILLIN RESISTANT
		I	II	III	UNCLASS-IFIED	NOT TYPABLE	
STUDENTS							
TEMPORARILY CONTAMINATED	508	23	9	22	6	41	43
CARRIERS	511	34	22	26	8	10	20
PERSONS IN GENERAL PRACTICE							
TEMPORARILY CONTAMINATED	161	26	23	24	7	20	28
CARRIERS	120	28	28	26	9	9	20
NURSES IN HOSPITAL							
TEMPORARILY CONTAMINATED	436	18	3	54	2	23	98
CARRIERS	391	26	10	52	2	9	94

Characters of strains of Staph. Aureus isolated from carriers and temporarily ~~contaminated~~ persons in the different population groups examined.

TABLE 37.

BACTERIOPHAGE GROUP	CARRIERS PERCENTAGE OF STRAINS NUMBER OF STRAINS	PENICILLIN RESISTANT	TEMPORARILY CONTAMINATED PERSONS PERCENTAGE OF STRAINS NUMBER OF STRAINS	PENICILLIN RESISTANT
I	161	17	115	16
II	102	6	44	8
III	156	33	110	67
UNCLASSIFIED	36	11	29	52
NOT TYPABLE	56	18	210	51
ALL GROUPS	511	20	508	43

Distribution of penicillin-resistant strains of Staph. Aureus isolated from carrier and temporarily contaminated students between the phage groups.

TABLE 38.

10. The Relationship of Antibiotic-resistant Carrier Strains of Staph. aureus to previous Antibiotic Therapy.

Many of the individuals who were examined for nasal carriage of Staph. aureus were questioned regarding previous antibiotic therapy. The results of *in vitro* antibiotic susceptibility tests were compared with previous antibiotic history. The results are shown in Table 39.

The proportion of the persons polled who admitted to previous penicillin therapy had increased so that in 1958 the large majority had experienced the antibiotic in some therapeutic form. The number of persons who have received chloramphenicol or the tetracyclines has increased in recent years and this may be reflected in the emergence of strains of staphylococci resistant to these antibiotics.

The proportion of nurses in hospital who had received penicillin was no greater than in the non-hospital population and as nearly all of these persons were carrying penicillin-resistant nasal staphylococci it is not possible to discern any relationship between therapy and antibiotic-resistance of the carrier strain in the group of the community .

Fifty-four per cent of persons questioned between 1951 and 1953 and seventy-eight per cent of those questioned in the years 1954 to 1957 had had previous penicillin therapy. In some this was probably minimal, such as the sucking of a few lozenges, while in others therapy may have included intense and prolonged courses of the antibiotic, but it was not found possible to /

to obtain reliable information to assess dosage more accurately.

Twelve per cent of those questioned in 1951-53 had had other antibiotics for therapy and this proportion increased to 20 per cent among those questioned between 1954 and 1957.

In the first period, 1951-53, 10 per cent of the carrier strains isolated from this group of people were penicillin-resistant and this had risen to 25 per cent in the second period, 1954-57. Less than 1 per cent of strains were resistant to antibiotics other than penicillin in the first period but more than 5 per cent were resistant during 1954-57.

More than 7 times as many penicillin-resistant strains were isolated from persistent carriers who had had previous penicillin therapy than from those who had not, and 4 times as many strains resistant to streptomycin, chloramphenicol and the tetracyclines were isolated from who had had one or more of these antibiotics than from those who had not.

After questioning persons in general practice who were found to harbour strains resistant to streptomycin, chloramphenicol or the tetracyclines, most were found either to have had the antibiotic, to have been in hospital, or have had contact with a person who had. (Table 40).

Thus there seems to be a close correlation between previous antibiotic therapy and the carriage of specifically-resistant strains of Staph. aureus. There is also however, a close association with hospitalisation, especially prolonged hospitalisation, and the harbouring of antibiotic-resistant strains in nurses and patients who have not received any antibiotic.

SOURCE OF STRAINS	NUMBER OF CARRIERS EXAMINED	PERCENTAGE WITH PREVIOUS EXPERIENCE OF PENICILLIN THERAPY	NUMBER WITH PENICILLIN-SUSCEPTIBLE CARRIER-STRAIN		NUMBER WITH PENICILLIN RESISTANT CARRIER-STRAIN	
			NO PREVIOUS EXPERIENCE OF PENICILLIN	AND PREVIOUS EXPERIENCE	NO PREVIOUS EXPERIENCE OF PENICILLIN	AND PREVIOUS EXPERIENCE
STUDENTS						
1950-1953	177	54	78	73	3	23
1954-1957	112	78	30	52	4	26
PERSONS IN GENERAL PRACTICE	120	60	55	40	5	20
NURSES IN HOSPITAL	116	66	8	8	42	58

Relationship between previous penicillin therapy and penicillin susceptibility of strains of Staph. Aureus isolated from persistent carriers.

TABLE 39.

	NUMBER OF CARRIERS WITH STRAINS OF Staph.Aureus IN THEIR NOSES RESISTANT TO		
	CHLORAAMPHENICOL	TETRACYCLINE	STREPTOMYCIN
NUMBER WITH PREVIOUS HISTORY OF THERAPY WITH CORRESPONDING ANTIBIOTIC.	3	3	5
NUMBER WITH HISTORY OF CONTACT WITH HOSPITAL.	2	0	17
NO PREVIOUS HISTORY OF ANTIBIOTIC THEREAPY OR HOSPITAL CONTACT.	0	1	6

Relationship of carrier strains of Staph.Aureus, isolated from persons in general practice and resistant to antibiotics other than penicillin, to previous antibiotic therapy or contact with hospital.

TABLE 40.

11. Carriage of Staph. aureus at sites other than the nose.

1: The work of Hare and his co-workers (Hare and Ridley, 1957) has emphasised that Staph. aureus may colonise other sites of the body than the anterior nares. Thus Staph. aureus may be recovered from swabs used to sample many areas of the body surface though most often the numbers cultured are very small and must indicate mere contamination of the surface. However on some occasions larger numbers of Staph. aureus are recovered and confirmed on repeated culture, so that establishment of the organism must have occurred.

The most frequently reported site of colonisation other than the nose has been the perineum (Hare, 1957,1958), but other flexures have been shown to yield the organism in large numbers and it seems that these sites are similar to the nose in being suited to the growth of the staphylococcus. Some investigators consider that Staph. aureus may grow in the bowel lumen so that faeces serves as a source of the organism. The phage type of the staphylococcus in the faeces often corresponds with that in the nose, and if it also corresponds with the perineal strain it may be that spread from the nose has occurred via the bowel. However the strain in the perineum may be different from the nasal strain, or the nose may be free of Staph. aureus when the perineum is colonised, so that it seems that the perineum can be a site of primary colonisation. Moreover the numbers

that /

that may be recovered are often more than can be explained by mere contamination. Therefore it must be accepted that independent colonisation of sites other than the anterior nares can occur.

Accordingly, a number of individuals were examined for carriage of Staph. aureus at several sites and the strains compared with those occurring in the nares.

2: Recovery of Staph. aureus from the Perineum, Axilla, Umbilicus and Chest Wall.

Sixty students were examined by swabbing various sites on the body and culturing for Staphylococcus aureus. Half of these persons were known to be persistent nasal carriers and the other half were known to have nares free of the organism.

Selective media were used to isolate the staphylococcus from the perineal cultures because of the large number of bowel and skin commensals also present which tended to overgrow the staphylococcus.

The results are shown in Table 40a.

Undoubtedly a number of persons were colonised with Staph. aureus at sites other than the anterior nares. In the majority of these persons colonisation of these sites was associated with colonisation of the anterior nares, but in a minority there was no colonisation of the nose. The importance of these persons is that they may be an undetected source of Staph. aureus for they are capable of disseminating the organism to their environment.

In /

In this connection a family examined in general practice was of interest. This family consisted of the mother, father and four children. The mother was free of Staph. aureus but the father and all the children were heavily colonised in the perineum without colonisation of their nares. The youngest of the children, aged 1 year also yielded large numbers of the organism from the umbilicus and axillae. This family was a potent source of the staphylococcus as was reflected in the heavy degree of contamination of their environment.

	NUMBER EXAMINED	NUMBER YIELDING MODERATE OR LARGE NUMBERS OF STAPH. AUREUS FROM THEIR			
PERSISTENT NASAL CARRIERS OF STAPH.AUREUS	30	AXILLAE 5	UMBILICUS 2	SKIN OF TRUNK 0	PERINEUM 10
NARES FREE OF STAPH.AUREUS	30	1	0	0	3
TOTAL	60	6	2	0	13

Isolation of Staph.Aureus from other sites on the skin of nasal carriers and persons with on staphycococci in the nose.

TABLE 40a.

STAPHYLOCOCCUS AUREUS
IN THE ENVIRONMENT

1: The number of persons harbouring Staph. aureus has been shown to be large in all sections of the community examined. These persons are likely to disseminate large numbers of the organism from the primary site of colonisation and will most certainly form a source of contamination of the environment. The environment thus contaminated will in turn form a reservoir for the recontamination of other persons who may or may not be established carriers.

Accordingly, quantitative measurements of the degree of contamination of the environment and the contribution from nasal carriers were made. Also a number of healthy nasal and perineal carriers were examined for dissemination of the staphylococcus and compared with persons who were known to be free of the organism in the nose and other flexures. Some individuals suffering from staphylococcal lesions were examined in a similar way.

2: METHODS OF MEASURING INDIVIDUALS FOR DISSEMINATION OF STAPH. AUREUS.

A: Surface sites on the body were swabbed as previously described.

B: Dissemination from the hands was simulated and measured in the following tests.

(1) Washing the hands in broth.

Five 20 ml. lots of nutrient broth were poured into the /

(1) continued

the cupped hands of the subject at intervals of 20 seconds. The movements of 'washing the hands' were performed and the broth washings collected in a sterile bowl.

The collected broth was vigourously stirred to distribute any micro-organisms and 1.0 ml. was diluted in 19 ml. of broth and well mixed. 0.2 ml. aliquots of the undiluted and diluted broth washings were spread over the surface of milk-agar plates by means of a sterile glass rod. After incubation colony counts were made and coagulase-positive staphylococci sub-cultured for further examination.

(2) Use of glass beads.

The transfer of organisms from the hands to a dry object was simulated by rolling 20 sterile, quarter-inch ballotini in the hands and fingers for 2 minutes, collecting the ballotini in a 1 oz. container and shaking with 20 ml. of broth. The surfaces of milk-agar plates were spread inoculated with 0.2 ml. aliquots of this broth.

(3) Touching an agar surface

Transfer /

(3) Continued

Transfer to wet objects was simulated by instructing the subject to touch the surface of sterile milk-agar plates with his hands and fingers. Two plates were used for each test, 'A' and 'B'. The palmar surface and fingers of the left hand were pressed on Plate 'A' and then on Plate 'B' ; next the palm and fingers of the right hand were pressed on Plate 'B' and secondly on Plate 'A'. In this way sampling errors were largely avoided and plates received approximately the same inoculum.

A sterile loop was used to spread the inoculum on Plate 'B' over the surface as evenly as possible and both plates were incubated. After incubation the colony counts on Plate A and B were compared.

The count on Plate B was higher if clusters of staphylococci were transferred to the surface of the plates as the mechanical action of spreading dispersed the agglomerates.

(4) Shaking hands.

A person with hands known to be free of Staph. aureus pressed their surfaces on an milk-agar plate. Next he shook hands with the subject/

(4) Shaking hands, continued

subject and pressed his hands on the surface of a second plate. A colony count after incubation revealed any staphylococci transferred during the shaking of hands.

C: Dissemination of Staph. aureus from the clothes.

This was measured by collecting dust produced by minor body movements carried out inside a sealed cabinet of 60 cubic feet capacity. (Fig.11)

The following movements were carried out by the subject at a normal rate and vigour, keeping the feet firm and avoiding speaking, coughing and sneezing. Each sequence took about 30 seconds and 10 sequences were performed in 10 minutes.

1: Remove pen from the jacket pocket, unscrew cap, replace cap and put the pen back in the pocket.

2: Pat jacket over the breast pocket to see if wallet safely in place.

3: Adjust tie using both hands.

4: Unbutton and rebutton one button of jacket front.

5: Loosen collar by sliding two fingers between the collar and back of the neck.

6: Bend over and touch shoe laces.

7: Put hand in trouser pocket and withdraw hand

8: Feel cuff-links or shirt sleeve buttons.

C: Dissemination of Staph. aureus from the clothes, Continued

The air in the cabinet was circulated for 30 seconds by means of a fan after the movements had been completed.

The air in the cabinet was sampled by means of a slit sampler sucking air through a $1\frac{1}{2}$ inch bore pipe attached to the side of the cabinet 3 feet from the ground, at a rate of 1 cubic foot per minute, onto suitable culture media in petri-dishes rotating once per minute.

The following sampling periods were used.

Control sample	Time of Sample 5 minutes before test	Duration minutes	Volume of air sampled
		5	5 cub. ft.
1st sample plate	during test period	5	5
2nd " "	1-6 minutes after test	5	5
3rd " "	7-12 " "	5	5
4th " "	13-23 " "	10	10
5th " "	24-34 " "	10	10
6th " "	35-60 " "	25	25
7th " "	90-120 " "	30	30

These sample plates were incubated and colony counts made, both of the total number of colonies on the plate and also the number of coagulase positive staphylococci. The Staph. aureus isolated were matched with the strains in the the nose or other primary site on the carrier.

D: Dissemination of Staph. aureus from the upper respiratory tract.

The following tests were carried out.

1: Examination of saliva. The subject was instructed to collect his saliva in the vestibule of the mouth and spit into a sterile basin. This saliva was thoroughly mixed in 100 ml. of broth and 1 ml. diluted to 100 ml. in broth. The surface of milk-agar plates were spread with 0.2 ml. aliquots of the undiluted and diluted saliva-broth and the number of colonies of Staph. aureus counted after incubation.

2: Sneeze

The subject gave one sneeze, or six simulated sneezes onto the surface of a 6 inch milk-agar plate held 12 inches in front of his mouth. Colony counts were made after incubation.

3: Air-borne droplet nuclei

A: One natural, or 6 simulated sneezes were delivered into the cabinet described in section B, from 6 inches in front of a cone attached to the door of the cabinet (see figure).

rig 12

3: Air-borne droplet nuclei, continued

The cabinet was sealed and sampling of the air carried out as described for 'body-movements' except that no samples were collected after 1 hour.

B: The dissemination of Staph. aureus in droplets produced by breathing was measured by instructing the subject to breathe for 10 minutes inside the cabinet without other movement and while dressed in a sterile boiler-suit which covered his whole surface except the head and face. (Fig 13)

Sampling of the air in the cabinet was carried out as previously described.

E: Dissemination from the handkerchief.

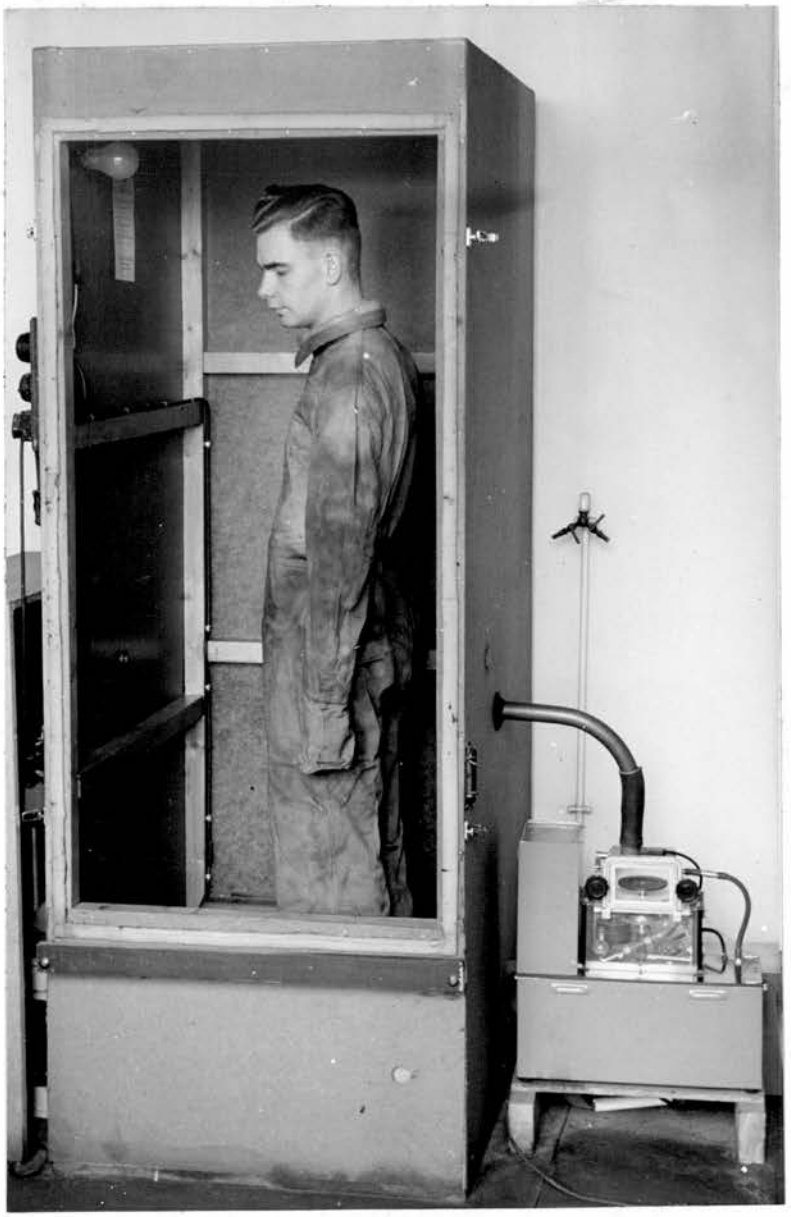
The subject was instructed to use a handkerchief for 5 days. For the test he entered the cabinet and stood still without speaking, coughing or moving. At each half-minute during the 5 minutes test the subject withdrew his handkerchief from his pocket and opened it for use, but did not touch his face and returned it to his pocket. After the test period the cabinet was sealed and the air sampled as previously described. Following incubation the sample plates were examined for Staph. aureus.

Cabinet for measuring dissemination of micro-organisms from carriers or patients with lesions. One of the slit-samplers is also shown, the other leading off from the other side of the cabinet.

FIGURE 11.

Cabinet with ' sneeze' extension fitted to its front. This allows a trajectory of nearly 6 feet for the droplets emitted. The candidate is dressed in a sterilised boiler suit to prevent organisms from his clothes contaminating the dust in the cabinet.

FIGURE 12.



Cabinet with candidate, clothed in sterile suit, in position for measuring dissemination of micro-organisms as a result of breathing. The door fits over the opening, providing an air-tight seal. Air may enter the cabinet via the filter hole in the lower right corner.

FIGURE 13.

RESULTS OF EXPERIMENTS TO MEASURE DISSEMINATION OF STAPH. AUREUS FROM HEALTHY PERSONS AND PATIENTS WITH SUPERFICIAL LESIONS

1: Dissemination from nasal carriers. Fourteen persistent nasal carriers of Staph. aureus were examined by the methods described above. The results are given in Table 41.

A: All of the fourteen carriers had nose swabs consistently positive for Staph. aureus though the number recovered from each carrier varied considerably as indicated.

B1: The number of Staph. aureus recovered from broth used to wash the hands of these carriers ranged from 10,000 -790,000, an average of 250,000 for 14 carriers.

B2: The number of staphylococci transferred to sterile beads ranged from 0-950 in 11 tests of 11 carriers, an average of 236 organisms per dry touch.

B3: Touching the wet surface of an agar plate transferred 3 - 400 Staph. aureus an average of 80 per wet touch in 13 carriers.

B4: The hand-shake transferred 0 -500 Staph. aureus to the clean hands of a non-carrier in 8 tests of 8 carriers, an average of 150 organisms per hand shake.

C: BODY MOVEMENTS. Eighty minor body movements were carried out in 5 minutes. Staph. aureus of the same phage type as found in the nose was liberated from 12 of 14 carriers. The numbers varied from 26-570 in positive experiments, and the average for the 14 carriers was 103 organisms for 5 minutes minor movements.

From the Graph (Fig. 14) it can be calculated that 90 per cent of these air-borne Staph. aureus were cleared in 26 minutes, that is 10 per cent remained air-borne; 99 per cent were cleared in 45 minutes leaving 1 per cent air-borne.

Staph. aureus accounted for 1.2 per cent of all the colonies on the milk agar plates.

D1: The saliva of 10 of the 14 carriers was free of Staph. aureus ; four carriers yielded 50 - 100 organisms.

D2: One to four sneezes were made per experiment and 28 experiments were carried on in 14 carriers. Staph. aureus was recovered in only 9 experiments in 3 carriers, in numbers up to 53 per sneeze with an average of 4.3 per sneeze. The Staph. aureus constituted about 0.01 per cent of all bacterial nuclei (about 40,000) capable of growing on the culture plates.

D3: Ten minutes breathing yielded an average of 7 Staph. aureus particles per 10 minutes if a snort was given each 30 seconds. Without snorts practically no organisms were recovered.

E: Use of a Handkerchief. In the test the handkerchief was used 10 times in 5 minutes and Staph. aureus was liberated into the air in all experiments with 13 carriers in numbers ranging from 12-1636 per use of the handkerchief, an average of 464 per use.

Ten per cent of these Staph. aureus remained airborne for 26 minutes, one per cent for 85 minutes, and some were still airborne after two and a half hours.

These particles of staphylococci constituted 0.6 - 25 per cent of all the bacterial particles liberated and capable of growth.

Five nurses who were nasal carriers were examined to compare their dissemination of Staph. aureus with those carriers already studied. The nurses were examined in uniform and were 'on duty' when sampled. The numbers of Staph. aureus liberated from their handkerchiefs and on their hands, or transferred during touching of surfaces, were comparable to those obtained from other nasal carriers. Fewer particles containing Staph. aureus /

Reference to test in text.	A	B1	B2	B3	B3	B4	C	D1	D2	D3a	D3b	E
SUBJECT	Numbers of Staph. Aureus on nose swab	Hand Wash in 100 Broth	Hand Touch Dry Beads	Hand Wet Plate 'A' Unspread	Touch Agar Plate 'B' Spread	Hand Shake Trans-fer	Body move-ments 5 minu-tes.	Saliva	Sneeze	Droplet Nucclei from Sneeze	Droplet Nucclei from Breathing	Handkerchief 1 Use
PA	++++	29,000	300	4	14	not tested	46	0	0	15	not tested	490
RO	++++	250,000	0	14	9	200	32	400	0	9	0	1636
GO	++++	250,000	12	44	30	58	152	0	0	5	19	723
McK	++	300,000	550	not tested	not tested	not tested	570	0	0	0	not tested	not tested
McG	+++	25,000	0	400	400	300	229	150	11	0	not tested	468
RAV	+++	136,000	8	40	300	500	107	0	2	0	1	367
DOU	++	10,000	50	80	40	0	0	0	0	0	0	12
ST	++	680,000	950	3	6	not tested	26	0	0	53	not tested	18
LAM	++++	40,000	250	28	3	not tested	110	0	0	1	not tested	204
GAR	+++	790,000	not tested	43	200	not tested	0	50	0	0	0	19
LEW	++	50,000	not tested	8	15	40	89	0	0	0	2	110
PH	++	175,000	not tested	79	150	18	47	0	0	0	0	747
AD	+++	100,000	11	26	35	88	30	0	0	0	0	759
HOW	++	100,000	8	8	10	not tested	22	1000	2	30	0	480

Estimated number of Staph.Aureus recovered in tests to measure dissemination from nasal carriers. The figures refer to organisms identical in phage type to the nasal strain.

TABLE 41.

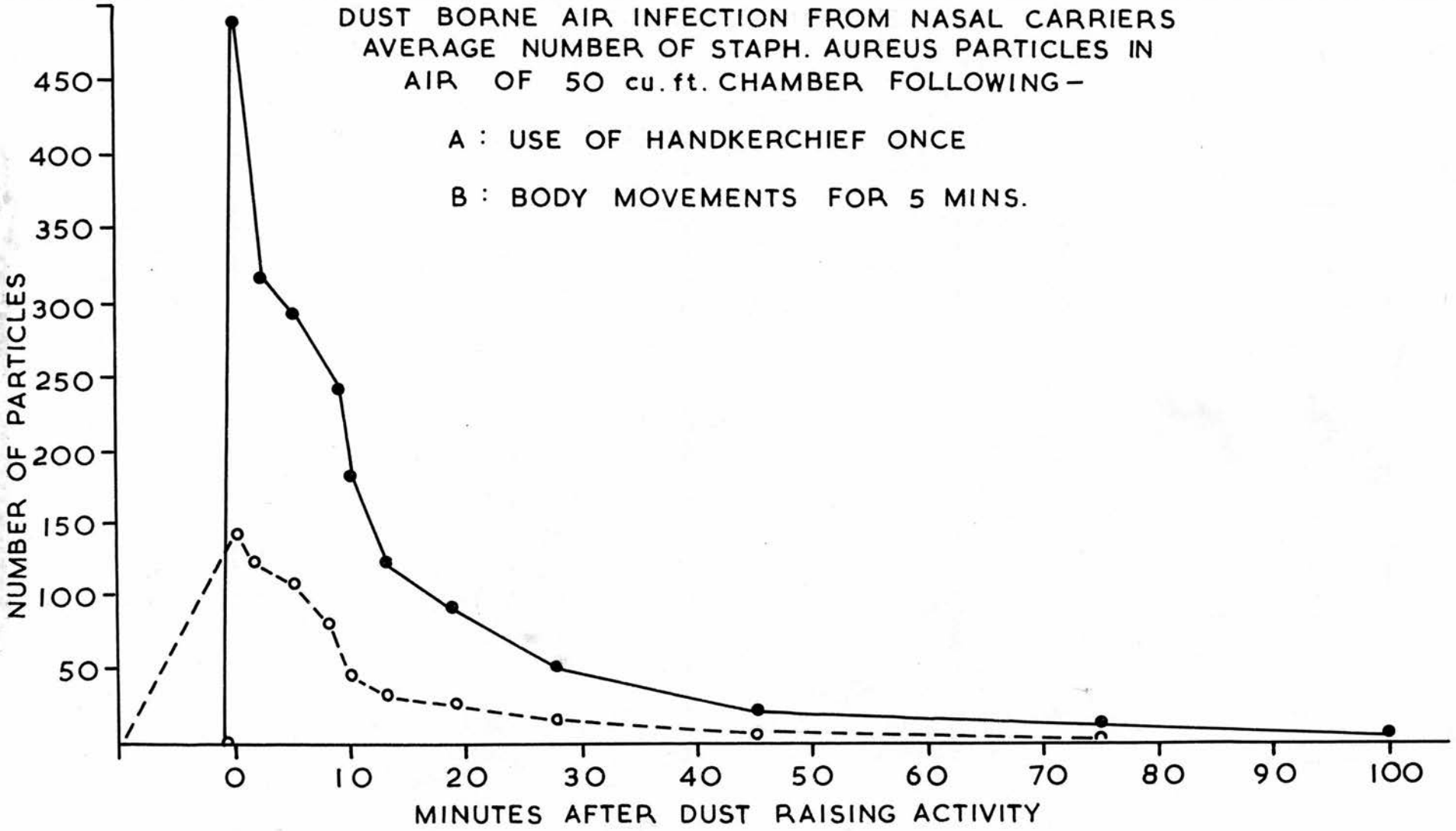

DUST BORNE AIR INFECTION FROM NASAL CARRIERS
AVERAGE NUMBER OF STAPH. AUREUS PARTICLES IN
AIR OF 50 cu. ft. CHAMBER FOLLOWING –
A : USE OF HANDKERCHIEF ONCE
B : BODY MOVEMENTS FOR 5 MINS.
NUMBER OF PARTICLES
450
400
350
300
250
200
150
100
50
0
10
20
30
40
50
60
70
80
90
100
MINUTES AFTER DUST RAISING ACTIVITY

FIGURE 14.

E: Use of a Handkerchief, continued

Staph. aureus were liberated from their clothing during body movements however, and this is probably related to the frequency with which the clothing is changed by these girls

Nurses. Staph. aureus was liberated in 4 out of 5 tests of 5 carriers in numbers ranging from 6 -100, the average being 30 for 5 minutes movements.

Staph. aureus was liberated in 5 out of 5 experiments of using the handkerchief in numbers ranging from 12-1240, an average of 354 per use of the handkerchief.

2: Dissemination from non-carriers. Similar experiments were carried out on 4 persons who were known to be non-carriers and persistently free of Staph. aureus and the results are given in Table 43. No Staph. aureus was liberated in 4 tests of 4 carriers. Staph. aureus was liberated in 2 of 4 experiments using the handkerchief in numbers ranging from 8-12 per use, an average of 10 particles per use of the handkerchief. No staphylococcal infected particles were liberated by breathing, sneezing or coughing and very few were transferred by means of touch.

It /

It is clear that these non-carriers were distributing many fewer Staph. aureus than nasal carriers.

3: Dissemination from Perineal Carriers. Three perineal carriers who were known to be free of Staph. aureus in the nose were examined. The results are in Table 44. Staph. aureus was liberated from them during body movements and transferred to objects via their hands in numbers comparable to the nasal carriers examined. Their handkerchiefs liberated 0 -80 particles, an average of 27 Staph. aureus particles per use.

4: Dissemination from patients with lesions.

Finally 8 persons who had or had recently had staphylococcal lesions were examined for dissemination of Staph.aureus. The numbers of the organism liberated during body movements, from using the handkerchief, and from their hands were comparable to the number recovered from nasal carriers(Table 45).

Referehce to test in text. SUBJECT	A Numbers of Staph. Aureus on nose swabs.	B1 Hand Wash in 100 Broth	B2 Hand Touch Dry Beads	B3 Hand-touch Wet-agar Plate 'A' Unspread	B3 Plate 'B' Spread	B4 Hand Shake Transfer	C Body move-ments 5 min-utes	D1 Saliva	D2 Sneeze	D3a Droplet Nucclei from Sneeze	D3b Droplet Nucclei from Breathing	E Hand-ker-chief 1 use
BR	-	100	0	2	2	not tested	0	0	0	not tested	nt	12
WH	-	0	0	0	0	not tested	0	0	0	not tested	nt	0
JO	-	1000	0	10	13	not tested	0	0	0	not tested	nt	8
DA	-	0	0	8	2	not tested	0	0	0	not tested	nt	0

Estimated number of Staph.Aureus recovered in tests to measure dissemination from non-carriers. The figures refer to any coagulase positive staphylococcus recovered.

TABLE 43

N1	~~++~~	20,000	nt	8	20	nt	12	nt	nt	nt	nt	368
N2	~~+++~~	8,000	nt	20	32	nt	34	nt	nt	nt	nt	112
N3	~~+++~~	6,000	nt	0	0	nt	100	nt	nt	nt	nt	40
N4	~~++++~~	100,000	nt	6	10	nt	6	nt	nt	nt	nt	1240
N5	~~++~~	2,000	nt	0	2	nt	0	nt	nt	nt	nt	12

Estimated number of Staph.Aureus recovered in tests to measure dissemination from nurse carriers. The figures refer to organisms identical in phage type to the nasal strain.
nt = not tested

TABLE 42

Reference to test in text	A	B1	B2	B3	B3	B4	C	D1	D2	D3a	D3b	E	A
SUBJECT	Numbers of Staph.Aureus on Perineal Swab	Hand Wash in 100 of Broth	Hand Touch Dry Beads	Hand-touch Wet-Agar Plate 'A' Unspread	Plate 'B' Spread	Hand Shake Transfer	Body Movements 5 mins.	Saliva	Sneeze	Droplet nuclei from sneeze	Droplet Nuclei from breathing	Handkerchief 1 use	Nose
NE	~~44~~	4000	8	0	0	not tested	220	nt	nt	nt	nt	16	-
SE	~~444~~	1000	4	6	20	not tested	164	nt	nt	nt	nt	0	-
BRI	~~444~~	10,000	20	30	28	not tested	46	nt	nt	nt	nt	80	-

Estimated number of Staph.Aureus recovered in tests to measure dissemination from perineal carriers. The figures refer to organisms identical in phage type to the perineal strain.

TABLE 44.

RE	~~444~~	10,000	nt	50	100	nt	219	nt	nt	nt	nt	0	604
LA	~~444~~	100,000	nt	200+	200+	nt	182	nt	nt	nt	nt	nt	420
PR	~~444~~	40,000	8	20	12	nt	32	nt	nt	nt	nt	0	180
M	~~444~~	2,000	0	nt	nt	nt	400	nt	nt	nt	nt	nt	1620
FOR	~~444~~	500,000	nt	60	100	nt	nt	nt	nt	nt	nt	nt	320
	~~44~~	150,000	20	8	12	nt	28	nt	nt	nt	nt	nt	760
	~~444~~	65,000	150	200	300+	nt	324	nt	nt	nt	nt	nt	128
	~~444~~	110,000	nt	nt	nt	nt	178	nt	nt	nt	nt	nt	960

Estimated number of Staph. Aureus recovered in tests to measure dissemination from persons with staphylococcal lesions.

nt = not tested

TABLE 45

4. THE DISSEMINATION OF STAPHYLOCOCCI BY CARRIERS

It is clear from the foregoing experiments that large numbers of Staph. aureus were liberated to the environment by the carriers though there was considerable variation in the numbers distributed by different individuals and from a few experiments completed, by the same individual at different times.

In the majority of nasal carriers the staphylococcus was not disseminated to any appreciable extent via the upper respiratory tract during the actions of breathing, speaking, coughing or sneezing. This is because in normal individuals the saliva will kill Staph. aureus and while the organism may be present in the nose and naso-pharynx it does not extend forward into the mouth. Unless there is forced snorting inappreciable numbers issue from the nose during breathing. Thus it is not surprising that face-masks have little influence on the spread of staphylococci from carriers (Forfar and Maccabe, 1958).

Spread from the nose was by (1) direct spread over contiguous surfaces to the face, the naso-pharynx and the gut ; (ii) transfer to the hands, and (iii) transfer to the handkerchief.

From the face the remaining surfaces of the body will be contaminated to a greater or lesser degree. Contamination of other parts of the body will also occur via the hands and the handkerchief. The clothes become contaminated from the hands and the handkerchief and eventually the outer garments will become /

become impregnated with the cocci. This was confirmed by the results of the experiments on body movements and the comparison with the numbers disseminated by the nurse carriers who had relatively clean outer garments on when they were examined.

Fomites and other persons will be contaminated directly by the hands of these carriers. The carriers examined in this investigation had large numbers of Staph. aureus on their hands, and appreciable numbers were transferred to the hands of an uncontaminated person during hand-shaking as well as to inanimate surfaces.

Air-borne dust was heavily and continually contaminated with the Staph. aureus particles from the carriers as was shown by the experiments on body movements and use of the handkerchief. Each carrier will daily contribute many thousands of viable Staph. aureus to the air. Many will remain air-borne, depending on their size, for many hours or even days if there is circulation of air currents. The larger particles will settle in the dust and be resuspended when the air is reagitated.

These infected particles will be inhaled by persons and contaminate their air passages and body surfaces, so that establishment and colonisation may result. Wounds and raw surfaces may be contaminated, and fomites will be continually exposed to contaminated dust settling on their surfaces.

If /

If the primary sites of colonisation of Staph. aureus are the perineum, umbilicus or the axillae the modes of spread of the organism will be similar. If the nose is free of staphylococci the numbers spread over the skin of the face and transferred to the hands and handkerchief will probably be less. Contamination of other parts of the skin surface will be heavier and the clothes will also become impregnated with the cocci.

The persons examined who had superficial staphylococcal lesions were not found to disseminate larger numbers of Staph. aureus than the carriers. However it is probable that there would be a greater degree of spread from lesions with more extensive discharge of exudate, and from infected lesions when the dressings were saturated with micro-organisms. However it has been noted that the numbers of Staph. aureus recovered from swabs which have sampled the anterior nares were comparable to the number recovered from many samples of pus. Therefore, a lesion may contaminate heavily for a short time, but a carrier contaminates continuously for a long time.

The source of Staphylococcus aureus were therefore the sites at which the organism was growing and multiplying, namely persons with lesions and healthy carriers. From these sources reservoirs in the environment received large numbers which would be related to the proportion of carriers and persons with lesions. To find out if this was so, experiments were carried out to measure the number of Staph. aureus in the environments of groups of persons already studied for carriage of the organism. The strains of staphylococci isolated

were /

were compared with those isolated from the carriers and from cases of infection.

STAPHYLOCOCCUS AUREUS IN THE ENVIRONMENT II. RECOVERY OF STAPH. AUREUS FROM THE ENVIRONMENT

1: Air-borne dust was sampled with a 'sieve sampler' previously described (page 84) and settling dust by means of settling plates. Blankets and other bedding and drapes were sampled by 'flap plates' and surfaces of fomites were swabbed.

The following environments were sampled.

1: HOSPITAL

(a) Surgical out-patient Department.

(b) Surgical theatres

(c) Surgical wards

(d) Corridors

(e) Medical wards

(f) Waiting Rooms

(g) Dispensary

(h) Maternity units

2: FACTORY

(a) Work floor

(b) Offices

3: UNIVERSITY DEPARTMENT

(a) No bacteriological work.

(b) Room used for bacteriological work

4: GENERAL PRACTITIONERS SURGERY

5 /

5: HOME ENVIRONMENT

(a) Household without infection

(b) Households with infection.

2: RESULTS The results of measuring the air-borne bacteria in these environments are shown in Tables 46 and 48. The total bacterial count was estimated on blood-agar plates, and the number of Staph. aureus from the more selective milk-agar plate cultures.

The total number of air-borne bacteria varied irregularly with each examination and depended on the degree of activity in the environment. It was never consistently higher in hospital environments than in non-hospital environments.

The number of Staph. aureus contaminated particles recovered from sites outside of hospital varied considerably. It was lowest in private houses where no known carriers or persons with lesions lived. It was considerably higher in houses where there were carriers and persons with staphylococcal infections. Most other situations examined had intermediate counts with the exception of the author's laboratory in which regular staphylococcal /

staphylococcal investigations were in progress.

Staph. aureus comprised about 0.1 per cent of the total bacteria sampled except in homes where no lesions or carriers occurred in which the proportion was very low, and the laboratory carrying out staphylococcal investigation in which the proportion exceeded 1 per cent.

The number of Staph aureus contaminated particles recovered from various sites inside hospital was consistently higher than from sites outside hospital. The greatest number were recovered from some surgical wards and maternity units. After a period of activity such as changing the babies, as many as 125 Staph. aureus contaminated particles per 100 cub. ft. were recovered from the changing rooms. Staph. aureus never comprised less than 0.1 per cent of the total bacterial count and sometimes exceeded 1 per cent. These values are greatly in excess of those obtained by Duguid and Wallace (1949) during similar experiments in hospital.

It is not possible to directly compare the results of the settling plate experiments with those of the air sampler. The majority of the particles sampled by the sieve-sampler are less than 25 microns in diameter. A higher proportion of the particles settling on exposed culture plates are larger.

It is interesting therefore to note that the relative proportion of Staph. aureus recovered from the settling plates and /

and sampler plates differed. This was particularly so in the hospital environment where Staph. aureus comprised 3 per cent of the infected particles settling in the Surgical Out-patient Department, and 7.5 per cent of those in the operating theatres examined. Thus in some of these theatres the total bacterial count was low, the number of air-borne Staph. aureus was not very high, but the number of large particles contaminated with Staph. aureus was relatively high. This may be of importance in the mechanism of the contamination of wounds in the theatre.

The number of Staph. aureus recovered from fomites, bedding and drapes in the various situations examined, corresponded roughly to the numbers of the organism present in the dust (Tables 47 and 49)

SITE	NUMBER OF EXAMS.	Staph. aureus per 100 c.ft.	Total bacteria per 100 c.ft.	Staph. aureus % of Total bacteria	Staph. aureus settling per sq.ft. per hr.	Total bacteria settling per sq.ft. per hr.	Staph. aureus % of Total bacteria
HOME							
No carriers or infections	2	0.1	1800	.005	0	220	0
Carriers. no infections	3	2.2	1650	.13	2	280	0.7
Carriers and infections	4	6.6	2800	.24	3	210	1.4
General Practitioner Surgery	3	3.0	3000	.1	1	180	0.5
UNIVERSITY							
Classroom	4	2.8	3100	.09	2	160	1.2
Laboratory No staphylococcal work.	3	1.5	2130	.07	1	200	1.0
Laboratory Staphyococcal work	6	20	1900	1.05	5	240	1.9
FACTORY							
General work floor	3	1.8	1700	.11	0	150	0
Offices	2	1.0	2460	.04	0	165	0

RECOVERY OF AIR?BORNE STAPH. AUREUS

FROM ENVIRONMENTS OUTSIDE HOSPITAL.

TABLE 46

SITE	NUMBER OF OBSERVATIONS	AVERAGE NUMBER OF COLONIES OF STAPH. AUREUS RECOVERED FROM		
		12 SWABS	IMPRESSION PLATE	FLAP PLATE
HOME				
No carriers or infections	2	2	Not tested	0
Carriers no infections	2	8	Not tested	1.8
Carriers and infections	3	18	Not tested	2.4
General Practitioner Surgery	2	13	2	Not tested
UNIVERSITY				
Classroom	2	6	0	Not tested
Laboratory with no Staphylococcal work	3	11	1	Not tested
Laboratory for Staphylococcal work	3	88	10	Not tested
FACTORY				
General work floor	2	12	Not tested	Not tested
Offices	2	7	Not tested	Not tested

RECOVERY OF STAPH. AUREUS FROM SURFACES

OF FOMITES IN ENVIRONMENTS OUTSIDE HOSPITAL

TABLE 47

TE	RECOVERED BY SIEVE SAMPLER: NUMBER OF EXAMINATIONS	STAPH.AUREUS PER 100 cub.ft.	TOTAL BACTERIA PER 100 cub.ft.	STAPH.AUREUS % OF TOTAL BACTERIA	RECOVERED BY SETTLING PLATES: STAPH. AUREUS SETTLING PER SQ.FT/NR	TOTAL BACTERIA SETTLING PER SQ. FT/NR	STAPH. AUREUS % OF TOTAL BACT
IDORS	3	7.2	2850	0.25.	4	250	1.6
TING ROOM	1	2.5	2300	0.1	0	180	0
PENSARY	2	5.4	1600	0.34	1	120	0.8
ICAL PATIENTS T.	8	12.0.	2140	0.56	3	100	3
ICAL OUT- IENTS T.	2	15.6	1800	0.85	3	160	1.9
GICAL DS	6	18.4	2560	0.7	6	200	3
ICAL D	2	13.5.	2920	0.45	2	150	1.3
RATING ATRES	7	17.4	1440	1.2	6	80	7.5
ERNITY TS	12	24.8	2780	0.9	5	140	3.6

Recovery of air-borne Staph.Aureus from hospital environments.

TABLE 48

SITE	NUMBER OF OBSERVATIONS	AVERAGE NUMBER OF COLONIES OF STAPH. AUREUS RECOVERED FROM		
		12 SWABS	IMPRESSION PLATE	FLAP PLATE
CORRIDOR	6	12	Not tested	Not tested
WAITING ROOM	3	2	Not tested	Not tested
DISPENSARY	2	4	0.25	Not tested
SURGICAL OUT PATIENTS	20	17	2	Not tested
MEDICAL OUT PATIENTS	2	8	Not tested	Not tested
SURGICAL WARD	12	35	3.6	4
MEDICAL WARD	10	22	1.8	2.5
OPERATING THEATRES	8	46	Not tested	Not tested
MATERNITY UNITS	24	110	4.5	4.6

RECOVERY OF STAPH. AUREUS FROM

FOMITES IN THE HOSPITAL ENVIRONMENT

TABLE 49

A STUDY OF STAPHYLOCOCCAL INFECTIONS

Staphylococcal infections result from the invasion and establishment in the host's tissues of Staphylococcus aureus which must be derived either from the host himself or from his environment, which includes other persons who are carriers.

A knowledge of the sources and routes of spread of the staphylococci causing infections is important for their efficient prevention. For this reason a study of strains of Staph. aureus isolated from staphylococcal infections occurring in persons in both the hospital and non-hospital communities was undertaken.

The strains of Staph. aureus were examined in the way already described as those from carriers and the environment. A general comparison of the strains isolated from carriers, lesions and the environment was made first and followed by more detailed experiments in which Staph. aureus was simultaneously isolated from carriers and lesions occurring in hospital and among the general community

1: Collection of strains of staphylococci from lesions

Strains of Staph. aureus were isolated from lesions occurring in hospital in-patients and out-patients. Many of these strains were obtained in sub-culture after isolation, through the courtesy of Drs. Bowie, Durie and Tonkin of the Bacteriology Department, Royal Infirmary ; Dr. Andrew Maccabe of the Northern Group Laboratory; Dr. Helen Wright of the University Laboratory ; Dr. P.N. Edmunds of the Southern Group Laboratory and Dr. Reid of Peel Hospital. The total number of hospital patients' lesion strains examined was 889.

A further series of 330 lesion strains was obtained from hsopital nurses suffering from staphylococcal lesions during the years 1956-58. These cases were seen by Dr. Verney.

Staphylococcal infections occurring in general practice were also examined through the courtesy of Dr. Donald Cruikshank, and from them 450 strains of Staph. aureus were available for examination.

Thus, a total of 1,700 strains from individual staphylococcal lesions were examined for antibiotic susceptibility and bacteriophage susceptibility.

2: Clinical Nature of the lesions

Examination of the lesions occurring in general practice confirmed that the overwhelming majority were superficial in nature comprising styes, skin pustules, boils, impetigo, sycosis barbae, infected abrasions, paronychia, whitlows and carbuncles. Only a small proportion involved deeper penetration of the tissues and were seen as cases of osteomyelitis, deep abscess, broncho-pneumonia, endocarditis and bacteraemia. (Table 50)

Nature of Lesions Examined

Per cent	Hospital Staff	Among Hospital Patients	Home Patients
Superficial	99	92	99
Deep	1	8	1

TABLE 50.

Lesions occurring among nurses in hospital were on the whole, clinically similar to those in general practice, the commonest being styes, pustules and boils, paronychia and septic fingers. Deeper abscesses and carbuncles were rare, and very few more serious lesions were observed.

The lesions among patients in hospital were divided into those which were present when the patient was admitted to hospital, or clearly contracted outside the hospital, and those which were undoubtedly contracted inside hospital. The majority of staphylococcal lesions seen in hospital out-patients were contracted outside hospital and contained many of the staphylococcal infections referred by general practitioners from general practice. Surgical operation wounds if infected were contracted while the patient was in hospital.

Most of the hospital contracted staphylococcal infections were clinically superficial, but there was a greater number of more deep seated infections occurring in hospital than outside in general practice. Many of these were infections of the respiratory or the urinary tracts some of which had bacteraemia or pyaemia. A few cases of staphylococcal enteritis also occurred. (Table 50).

The patient in hospital was more liable to contamination with Staph. aureus than the patient outside hospital as shown by the figures for the number of staphylococci in the different environments (Section p.177). The hospital patient was also more /

more likely to be liable to the establishment of infection than the normal healthy individual because he might either be suffering from disease which reduced the efficiency of his defence mechanisms, so allowing staphylococci to invade his tissues, or he might have had his surface defence mechanisms artifically breached by surgical trauma so that underlying tissues were exposed to contamination with which they do not normally have to contend. Also, those patients receiving antibiotics for prophylactic or therapeutic purposes are more favourable hosts to antibiotic-resistant micro-organisms since their tissues contain the antibiotic which acts as a selective agent and the reduction of their normal commensal flora facilitates access of contaminating bacteria.

3: Results of examining strains of Staph. aureus isolated from lesions for susceptibility to bacteriophage and antibiotics

There was a difference between the strains of Staph. aureus isolated from hospital patients and those isolated from patients outside hospital both in the distribution of bacteriophage patterns and susceptibility to antibiotics. These were similar to the differences described between the nasal carrier strains isolated from persons in hospital and those outside hospital (Table 51).

This difference was even more marked when strains derived from hospital patients lesions contracted outside hospital were excluded from the hospital strains. (Table 52).

Broadly speaking, strains of Staph. aureus from hospital lesions were penicillin-resistant, and the majority had bacteriophage patterns belonging to Group III ; in contrast strains isolated from lesions in general practice were predominantly penicillin-susceptible, and strains with bacteriophage patterns of Group III much less frequent.

When these strains were compared with those isolated from nasal carriers in the respective environments a marked similarity was obvious (Figs15 16) which suggests that there is some relationship between nasal carrier strains and infections. This was investigated in more detail in the following experiments.

SOURCE OF STRAINS	NUMBER EXAMINED	PERCENTAGE WITH BACTERIOPHAGE PATTERNS OF GROUP					PERCENTAGE OF STRAINS PENICILLIN RESISTANT
		I	II	III	Unclass -ified	Not typable	
PATIENTS IN HOSPITAL 'R'	340	36	12	38	5	9	70
HOSPITAL 'A'	40	32	5	45	7	10	85
HOSPITAL 'E'	120	20	6	68	0	6	82
HOSPITAL 'W'	80	43	5	46	6	0	81
HOSPITAL 'P'	38	46	3	45	6	0	92
HOSPITAL 'L'	271	24	14	57	5	6	72
All hospital patient strains	889	31	11	48	3	7	75
LESIONS IN GENERAL PRACTICE	450	29	23	28	13	7	22
Infections in Hospital Staff	330	47	8	36	0	9	96

CHARACTERS OF STRAINS OF STAPH.AUREUS ISOLATED FROM 1700 LESIONS OCCURRING IN HOSPITAL AND GENERAL PRACTICE.

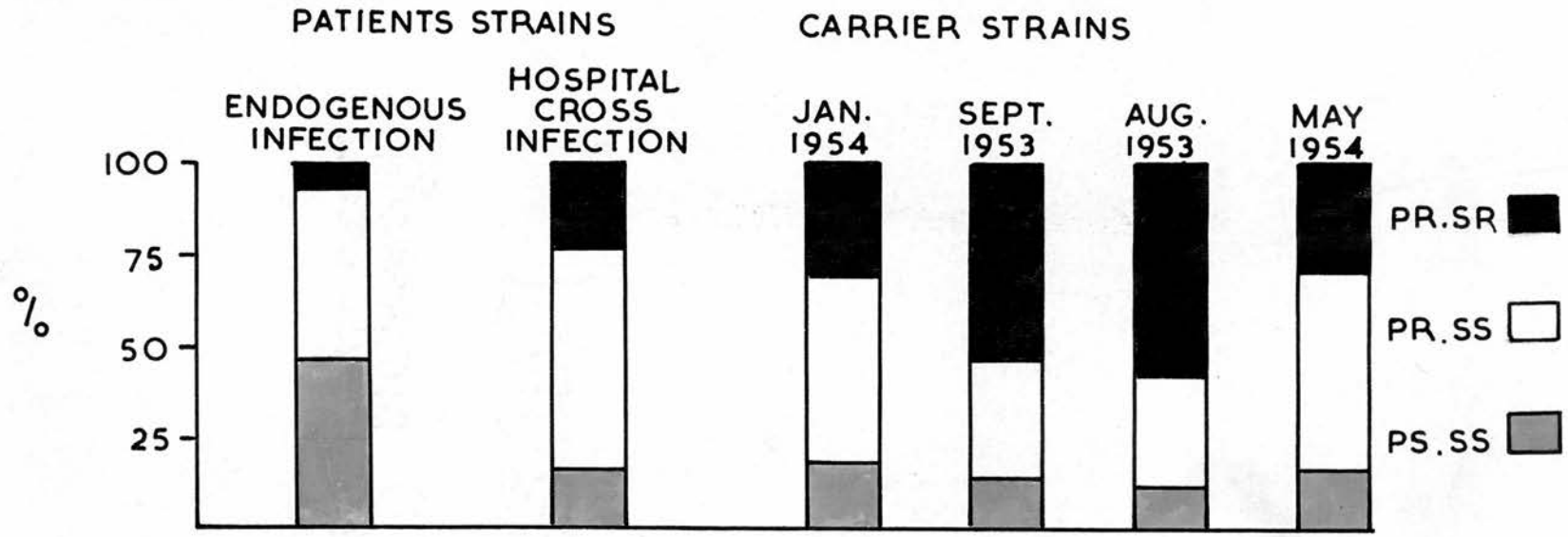
TABLE 51

SOURCE OF STRAINS	NUMBER EXAMINED	PERCENTAGE WITH BACTERIOPHAGE PATTERN OF GROUP					PERCENTAGE OF STRAINS PENICILLIN RESISTANT
		I	II	III	Unclass -ified	NT *	
OUTPATIENTS IN							
HOSPITAL 'R'	138	40	19	31	3	7	44
HOSPITAL 'L'	114	32	27	35	0	6	55
BOTH HOSPITALS	252	37	24	33	1	5	49
INPATIENTS							
HOSPITAL 'R'	202	33	8	42	6	11	88
HOSPITAL 'L'	157	20	8	68	0	4	83
BOTH HOSPITALS	359	27	8	54	3	8	86

* NT = Not typable.

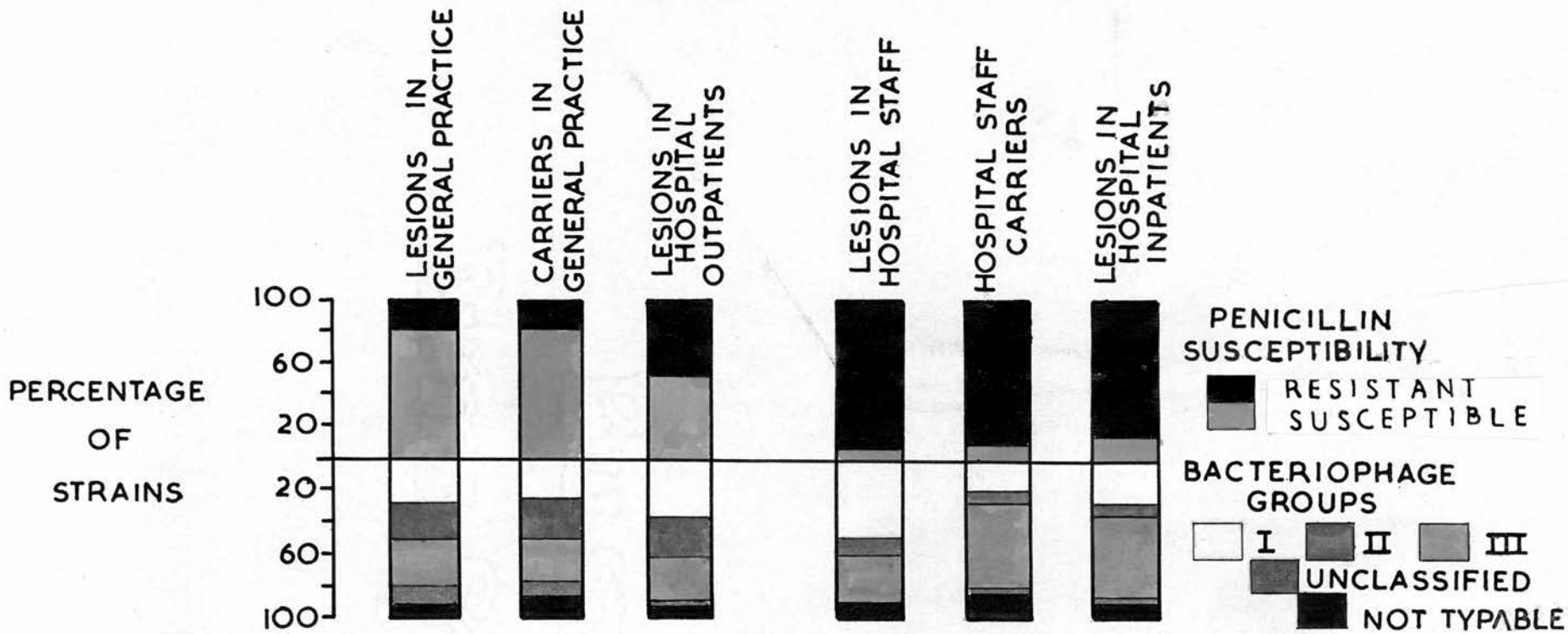
CHARACTERS OF STRAINS OF STAPH. AUREUS ISOLATED FROM LESIONS IN OUTPATIENTS AND INPATIENTS OF TWO HOSPITALS.

TABLE 52



ANTIBIOTIC SENSITIVITY OF STRAINS OF STAPHYLOCCUS PYOGENES ISOLATED FROM PATIENTS AND CARRIERS

FIGURE 15.



COMPARISON OF STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM CARRIERS AND LESIONS IN HOSPITAL AND GENERAL PRACTICE

FIGURE 16.

AN INVESTIGATION OF THE SOURCE OF STAPHYLOCOCCAL INFECTIONS.

The source of the staphylococci producing infections may be any site of growth of the organism. The most obvious are open lesions in patients and persistent carriers.

Open lesions are likely to be a more common source in hospital for the chances of cross-infection are greater. The importance of lesions as a source will be less in the home environment because there the chances of cross-infection are less.

The observations already made show that there is a similarity between the strains isolated from persistent carriers and lesions in the same group of persons. It is therefore possible that carriers are an important source of stphylococci, producing lesions both in the hospital and non-hospital communities.

To investigate this problem two experiments were carried out to determine the relationship between the strains present in carriers and their environment, and those in patients with lesions. These experiments were carried out both inside and outside hospital because of the marked difference in the antibiotic sensitivity and bacteriophage types of Staph. aureus in these two environments.

The first experiment was possible because of an invitation to investigate the cause of sporadic outbreaks of staphylococcal infection in Leith Hospital. My observations, assisted for part of the time by Mr. I. C.S. Knight and Dr. W.S.A. Allan, extended over nearly two years.

The second experiment was designed to take place in an urban general practice with the help of the general practitioner, Dr. Donald Cruikshank. This work has extended over 4 years.

AN INVESTIGATION OF STAPHYLOCOCCAL INFECTIONS IN HOSPITAL

In the hospital in which this investigation took place a number of cases of surgical wound infection were occurring sporadically, and on routine bacteriological examination Staphylococcus aureus was isolated from a large proportion. The strains of staphylococci isolated from the lesions frequently appeared to be similar in their coagulase and pigment production, and in their susceptibility to antibiotics. This suggested that the infections might have a common source.

The entire nursing staff, the majority of the graduate medical staff and many of the ancillary staff, were examined for nasal carriage of Staph. aureus at monthly intervals. During this period all infections in the hospital were examined, and material from them submitted for bacteriological examination. At intervals bacteriological examination of the hospital environment was carried out by sampling air-borne dust and fomites.

1: Nasal Carriage Rate of Staph. aureus.

The carriage rate among the hospital staff was 31 per cent during the summer of 1953 (Table 53); this rate gradually increased until it was 42 per cent in the middle of winter.

At the same time the opportunity was taken of sampling nurses who came to start work in the hospital. Twenty-nine per cent of these nurses were nasal carriers. A number had previously worked in other hospitals and were not regarded as 'new' to the hospital environment. The others were followed up to see whether they became colonised with the hospital strains. /

strains. This was possible because of the differences in the antibiotic and bacteriophage susceptibility of the hospital strains of Staph. aureus and those brought in from outside the hospital.

2: Antibiotic susceptibility of the staphylococci isolated from nasal carriers among hospital staff.

The minimum inhibitory concentrations of the 5 antibiotics most frequently used in the hospital at the time, penicillin, streptomycin, chloramphenicol, chlorteracycline and oxytetracycline were determined for each strain. In this particular hospital it was found that the strains of staphylococci could be classified into three groups according to their susceptibility to penicillin and streptomycin. Thus there were the patterns,

Penicillin-susceptible and streptomycin-susceptible PS. SS.

Penicillin-resistant and streptomycin-susceptible PR. SS.

Penicillin and streptomycin resistant PR. SR.

No strain resistant to chloramphenical or the tetracyclines was isolated from the carriers.

3: Bacteriophage susceptibility of the carrier strains.

Eighty per cent of the carrier strains had bacteriophage patterns belonging to Group III. Most of the Group I and II strains were isolated from staff who were newcomers to the hospital and these strains were more frequently penicillin susceptible. (Table 54).

4: Incidence of infections

A /

4: Incidence of Infections

A total of 231 cases of staphylococcal infection were examined in the hospital between January 1953 and February 1954. During the first half of 1953 the number of cases occurring each month was more or less constant but during the third quarter of the year there was a marked fall followed by a rise to the original level during the last quarter. (Table 55).

A proportion of these infections were clearly contracted before coming to hospital, for example boils and carbuncles, breast abscesses and some cases of respiratory and urinary tract infection. The strains of staphylococci isolated from these cases were more often sensitive to penicillin and had different patterns of susceptibility to bacteriophages, most of the strains belonging to Groups I and II. (Table 56).

The remaining cases were those which almost certainly were contracted in hospital, comprising infections of operation wounds, varicose ulcers, burns and urinary and respiratory tracts in adults and the skin and eyes. The strains of staphylococci isolated from these cases were nearly all resistant to penicillin, and many were also resistant to streptomycin. The great majority had bacteriophage patterns belonging to Group III. These strains thus conformed closely to the strains isolated from the hospital staff carriers in phage 'type' and antibiotic susceptibility. (Fig. 13) (Table 56) .

NUMBER OF STRAINS WITH		NUMBER OF STAFF REGULARLY EXAMINED	NUMBER FROM WHOM LARGE NUMBERS OF STAPH. AUREUS WERE ISOLATED IN						
			MAY	JULY	SEPT.	NOV.	DEC.	JAN.	JUNE
		116	38	38	40	47	38	35	28
BACTERIOPHAGE PATTERN BELONGING TO GROUP	I	5	5	5	5	6	5	4	6
	II		4	3	3	4	4	4	5
	III		29	30	32	37	29	27	17
ANTIBIOTIC SUSCEPTIBILITY PATTERN.	PS:SS		7	7	8	7	8	7	6
	PR:SS		20	21	23	28	20	16	14
	PR:SR		11	10	9	12	10	12	8

NASAL CARRIAGE RATE AMONG STAFF OF LEITH HOSPITAL DURING 1953-54 AND ANTIBIOTIC AND BACTERIOPHAGE SUSCEPTIBILITY OF STRAINS OF STAPH. AUREUS ISOLATED.

TABLE 53

DATE	NUMBER OF CARRIER STRAINS	NUMBER OF STRAINS PENICILLIN SENSITIVE	WITH PHAGE PATTERNS OF GROUP I	II	III	NUMBER OF STRAINS PENICILLIN RESISTANT	WITH PHAGE PATTERNS OF GROUP I	II	III
MAY 1953	38	7	3	3	1	31	0	2	29
JULY	40	8	2	4	2	32	1	1	30
SEPT.	38	8	3	4	1	30	2	2	26
DEC.	35	7	2	3	2	28	2	1	25
FEB.1954	35	7	3	2	2	28	2	1	25
JUNE	28	6	2	2	2	22	4	3	15

BACTERIOPHAGE GROUPS OF PENICILLIN-SUSCEPTIBLE AND RESISTANT STRAINS OF STAPH.AUREUS ISOLATED FROM LEITH HOSPITAL STAFF CARRIERS.

TABLE 54.

DATE	TOTAL NUMBER OF INFECTIONS EXAMINED	NUMBER OF INFECTED OPERATION WOUNDS	NUMBER OF OTHER HOSPITAL INFECTIONS	NUMBER OF NON-HOSPITAL INFECTIONS
JAN. 1953	22	10	2	10
FEB.	22	9	4	9
MARCH	20	8	4	8
APRIL	17	5	7	5
MAY	17	6	5	6
JUNE	14	6	2	6
JULY	9	3	3	3
AUGUST	8	3	2	3
SEPT.	15	5	5	5
OCT.	18	5	8	5
NOVEMBER	14	7	5	2
DECEMBER	15	6	4	5
JAN.1954	18	8	5	5
FEB.	22	9	4	9
Total	231	90	58	83

MONTHLY INCIDENCE OF STAPHYLOCOCCAL INFECTIONS

EXAMINED IN LEITH HOSPITAL FROM JANUARY 1953-FEBRUARY 1954.

TABLE 55.

SOURCE OF STRAINS	NUMBER OF STRAINS	PERCENTAGE OF STRAINS WITH BACTERIOPHAGE PATTERN OF GROUP				WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN		
		I	II	III	NT*	PS:SS	PR:SS	PR:SR
HOSPITAL INFECTIONS	148	20	8	68	4	17	59	24
Non-HOSPITAL INFECTIONS	83	32	27	35	6	47	49	4
HOSPITAL CARRIERS	82	19	6	75	0	13	54	33

* NT= Not typable.

ANTIBIOTIC AND BACTERIOPHAGE SUSCEPTIBILITY OF STRAINS OF STAPH. AUREUS ISOLATED FROM LESIONS EXAMINED IN LEITH HOSPITAL BETWEEN JANUARY 1953 AND FEBRUARY 1954.

TABLE. 56.

5: Comparison of Strains of Staph. aureus isolated from carriers and cases of hospital infection.

The antibiotic susceptibility and bacteriophage groups of the two groups of strains were closely similar. Examination of individual strains showed that the majority were identical. Thus two strains were most frequently isolated from the carriers in the hospital and the same two strains were those most commonly isolated from the cases of infection. The two strains were :

1: Phage pattern 70/76/77 Antibiotic susceptibility pattern PR. SR. CS. TS.

2: Phage pattern 47/53/54/75/76/77 and susceptibility PR. SS or SR. CS. TS.

The frequency of the other strains isolated from the carriers and infections, with details of their bacteriophage patterns and antibiotic susceptibility are shown in Table 57.

6: Nature of strains of Staph. aureus recovered from the nares of patients with hospital staphylococcal lesions.

Unfortunately the patients' nares were not regularly swabbed and therefore only a limited number of nasal cultures were available for examination. A number of the swabs that were taken sampled the patients after they had been in hospital several weeks with infection, and this must have had an influence upon their nasal flora.

From /

From the results that are available (Table 58) it was obvious that few of the patients with hospital contracted lesions could have been the source of their own infections.

7: Staphylococcus aureus in the dust, air and bedding of the hospital.

Several examinations of dust from the air, bedding, fomites and floors in the hospital were made. A number of staphylococci were recovered and some of these were coagulase positive, though a relatively small number were similar to the strains isolated from the nasal carriers or the lesions in patients. Many of the dust strains were not typable with the stock phages. The majority of the dust strains of staphylococci were penicillin-resistant and some were also resistant to streptomycin.

8: Comparison of the strains of staphylococci isolated from carriers and lesions.

The strains of Staph. aureus isolated from the nasal carriers and the cases of hospital infection may be compared on the basis of their antibiotic susceptibility and bacteriophage susceptibility patterns.

All of the hospital carrier strains were lysed by the phages, and more than 75 per cent had patterns belonging to Group III.

The /

The remaining strains with patterns of Groups I and II were most frequently isolated from nurses who had recently arrived to work in the hospital. The large majority of the Group III strains belonged to two distinct patterns or 'types', "70/76/77" and "47/53/54/75/76/77", the first most frequently resistant to both penicillin and streptomycin, the second almost always resistant to only penicillin. However the antibiotic susceptibility of these strains was not constant.

The majority of the strains isolated from patients with infections contracted in hospital, were similar in phage type and antibiotic susceptibility to those isolated from the carriers. Thus the most frequently occurring strains in the infections were 70/76/77 and 47/53/54/75/76/77. The identity and frequency distribution of these strains was similar in both carriers and cases and so strongly suggest that there was a causal relationship between the two. No such relationship existed between the Staph. aureus isolated from the patients' own nares and their lesions, and the small numbers of strains of staphylococci isolated from dust, which were similar to those in the lesions suggests that dust was causally less important than the carriers.

Strains of Staph. aureus isolated from patients with lesions contracted outside hospital were quite different from the strains in hospital staff carriers and hospital lesions. The majority were susceptible to penicillin, and the phage 'types' many and varied, thus corresponding to the strains of staphylococci isolated from the general population.

Staff carriers					Hospital contracted infections					Infections contracted outside hospital				
Number of carriers	Strain				Number of lesions	Strain				Number of lesions	Strain			
	Bacterio-phage pattern	Antibiotic susceptibility PS:SS	PR:SS	PR:SR		Bacterio-phage pattern	Antibiotic susceptibility PS:SS	PR:SS	PR:SR		Bacterio-phage pattern	Antibiotic susceptibility PS:SS	PR:SS	PR:SR
13	70/76/77	0	3	10	16	70/76/77	0	2	14	1	6/7/54/77			1
12	47/53/54/ 75/76/77	1	10	1	10	47/53/54/ 75/76/77	0	10	0	1	54/77		1	
2	7/44	0	2	0										
1	44/47	0	0	1										
1	47/47A	0	1	0	1	47/47A/54		1		1	47/47A/ 47C	1		
1	52	0	1	0						1	29	1		
3	52/52A	2	1	0	4	52/52A	2	2		2	52/52A	1	1	
1	44/70	1	0	0	1	52A			1	1	42B/52/ 73	1		
1	3A/3B/3C	1	0	0	1	3B/3C/51	1			2	3A/3B/3C	2		
2	3A/3B	2	0	0	1	42E			1	2	3A/3B/3C/ 51/55	2		
1	3A/51	1	0	0						1	3A/3B	1		
					1	N.T.			1	1	3B/3C/51	1		

Details of strains of Staph. aureus isolated from hospital staff nasal carriers and patients with staphylococcal lesions between October and December, 1953

TABLE 57.

NUMBER OF PATIENTS EXAMINED FOR NASAL STAPHYLOCOCCI	NUMBER POSITIVE FOR STAPH. AUREUS	NUMBER WITH VERY SMALL NUMBER OF STAPH. AUREUS
28	10	3

DETAILS OF PATIENTS'

NASAL STRAINS		LESION STRAINS
BACTERIOPHAGE PATTERN	ANTIBIOTIC SUSCEPTIBILITY PATTERN	BACTERIOPHAGE PATTERN
3a/3b/3c	PS:SS	70/76/77
3c/51/55 42D	PS:SS	47/53/54/75/76/77
	PS:SS	47/47a/53/54
42e/79	PS:SS	70/76/77
29/52	PS:SS	52/52a
52/52a	PR:SS	70/76/77
52/52a	PS:SS	3b/3c/51
6/7/47/75	PS:SS	47/53/54/75/76/77
7/47 +	PR:SS	47/53/54/75/76/77
70/76/77	PR:SR	70/76/77

STRAINS OF STAPH. AUREUS ISOLATED FROM ANTERIOR NARES OF PATIENTS WITH STAPHYLOCOCCAL LESIONS.

TABLE 58.

SOURCE	NUMBER OF STAPH. AUREUS COLONIES RECOVERED	NUMBER WITH BACTERIOPHAGE PATTERN		ANTIBIOTIC SUSCEPTIBILITY PATTERN
		No.	Pattern	
DUST	54	12	70/76/77	PR:SR
		9	47/53/54/75/76/77	PR:SS
		3	3a/3b/3c	PR:SS
		1	42b/73	PS:SS
		9	Other patterns	PS & PR
		20	Not typable	PS & PR
BEDDING	26	5	70/76/77	PR:SR
		3	47/53/54/75/76/77	PR:SS
		4	52/52a	PR & PS
		2	Other patterns	PS
		12	Not typable	PS & PR
FOMITES	37	3	70/76/77	PR:SR
		10	47/53/54/75/76/77	PR:SS &SR
		2	3a/3b/3c	PR & PS
		1	52/52a	PS:SS
		8	Other patterns	PS & PR
		13	Not tyable	PS & PR

RECOVERY OF STAPH. AUREUS FROM AIR-BORNE DUST,

SURFACE OF FOMITES AND BEDDING IN LEITH HOSPITAL.

TABLE 59.

B. STAPHYLOCOCCAL INFECTIONS IN GENERAL PRACTICE

This investigation into staphylococcal infection was carried out in a non-industrial and urban practice. All the patients were seen by one partner so that clinical assessment was as uniform as possible.

The investigation was confined to non-injury lesions, and with the exception of two cases of osteomyelitis and two deep abscesses, which were contracted in hospital, all the infections were clinically superficial. All the patients who reported with lesions were examined clinically and bacteriologically.

Swabs were taken from the anterior nares, from the lesion exudate when available, from the skin overlying the lesion and from the skin at other sites on the body. The cultures from these swabs were examined for staphylococci.

Nasal carriage rate in the practice

The nasal carriage rate of Staph. aureus had already been estimated in a survey of this practice. The results of sampling 300 persons in the practice known to be free of staphylococcal lesions showed a carriage rate of 33 per cent. (Table A 60)

Number of persons examined	percentage persistent carriers	percentage temporarily contaminated	percentage penicillin sensitive	percentage of carrier strains of bacteriophage group				
				I	II	III	UnC	NT
300	33	13	84	24	37	14	6	19

Nasal carriage rate in general practice and details of carrier strains of Staphylococcus aureus.

Table A 60

2: Nature and incidence of Staphylococcal Lesions.

In the four years under review (1955-1958), 376 individual cases of staphylococcal infection were seen, and the number of episodes of infection was 791. This represents an infection rate of approximately 5 per cent of the persons in the practice. There were 214 male and 162 female cases which corresponds to the sex distribution in the practice. No particular age group was involved, lesions being seen in new-born infants and in aged persons. There was no marked seasonal increase or decrease in the occurrence of lesions. No particular social or occupational group of the community in the practice were found to have more lesions than another.

Two-thirds of the lesions seen were boils or septic spots ; 19 per cent were styes and 2 per cent were abscesses. Septic lesions of the hands and feet comprised 11 per cent and only 1 per cent were more serious 'Deep' lesions, two being cases of osteomyelitis. The distribution of these lesions on the body is shown in Table 60a.

Fifty-four per cent of the patients reporting with a lesion had a history of previous lesions within two years and were regarded as cases of recurrent infection. Only 19 per cent of the patients had no history of previous lesions at some time in their lives.

Three-hundred and seventy six patients with lesions were examined.

Eighty-four per cent of the 326 patients were persistent nasal carriers of Staph. aureus. The organism was not isolated from the nares of 52 but in 15 systemic administration of antibiotic shortly before probably affected the isolation of the staphylococcus, and in one, previous radiation therapy for cancer is likely to have had the same effect. In some of the remainder it is possible that other flexures such as the perineum were colonised with Staph. aureus but these sites were not regularly examined.

In 92 per cent of the persistent nasal carriers with lesions the same phage type of organism was isolated from both sites. In 22 cases, organisms of different phage type were isolated from nose and lesion, and in 14 Staph. aureus was isolated from the lesion but not from the nose. (Table 61)

Staph. aureus was isolated from 84 per cent of the 791 lesions; in 46 cases failure to isolate the organism was due to the lesion not discharging, and it is of interest to note that in only 11 per cent was the organism isolated from the skin overlying the lesion in these patients.

Number of cases of Staphylococcal infection	376
Number with recurrent infection	204
Number of cases in whom Staph. aureus isolated from lesion	326
Number of these cases with Staph. aureus in the nose in large numbers	274
Number of cases in which nasal and lesion organism identical	251
Number with nose free of Staph. aureus	52

Isolation of Staph. aureus from noses and lesions of cases of Staphylococcal infection occurring in general practice.

Table 61.

3: Antibiotic and bacteriophage susceptibility of strains from lesions

Seventy-five per cent of the lesion strains were sensitive to penicillin, producing no penicillinase. These strains comprised a large number of distinct bacteriophage 'types' which belonged predominantly to Groups I and II. (Table 62)

The remaining 25 per cent of strains were penicillinase producers but were evenly distributed through the different phage groups. Only a very small number were resistant to streptomycin, chloramphenicol or the tetracyclines. No strain was resistant to erythromycin, neomycin, bacitracin, polymyxin or novobiocin.

4: Comparison of strains of staphylococci isolated from carriers and lesions.

The proportion of persistent nasal carriers of Staph. aureus in the practice investigated was similar to that usually found among the non-hospital population. The observations in this investigation showed that the majority of infections occurred in these persistent nasal carriers and were due to staphylococci identical with these harboured in their nares. From this it can be concluded that infection in most cases was endogenous and the source was the patients' own nose.

In a minority of cases, where there was no evidence of nasal carriage, or where the staphylococci in the lesion were not the same as in the nose, it was likely that the infection had an exogenous source.

The staphylococci concerned in these infections in general practice were very different from those found in the infections examined in hospital. Phage typing showed that a very large

number /

number of different strains were concerned and in most cases the strain was identified with the patient ; this is also in contrast to hospital studies where a limited number of strains were responsible for the infections. In addition nearly all the hospital strains were penicillin-resistant and frequently resistant to the other antibiotics, but the strains observed in general practice were predominantly sensitive to penicillin and very rarely resistant to other antibiotics.

SITE OF LESION	NUMBER	PERCENTAGE OF TOTAL	
HEAD	8	2	
NOSE	38	10	HEAD AND NECK
FACE	46	12.5	60%
NECK	52	14	
EYES	76	21	
AXILLA	14	3.5	
ARMS	20	5.5	UPPER LIMBS
HANDS	37	9.5	19%
ABDOMEN	3	0.5	
CHEST	12	3	TRUNK
BACK	12	3	13%
BUTTOCK	18	4.5	
VULVA	6	1.5	
ANUS	2	0.5	
LEGS	29	8	LOWER LIMBS
FEET	2	0.5	8%

SITE OF PRESENTING LESION IN 376 CASES OF STAPHYLOCOCCAL INFECTION OCCURRING IN GENERAL PRACTICE.

TABLE 60.

SOURCE OF STRAINS	NUMBER OF STRAINS	PERCENTAGE OF STRAINS PENICILLIN SENSITIVE	WITH BACTERIOPHAGE PATTERNS OF GROUP I	II	III	Unclassified	NT*
CASES WHERE NOSE AND LESION STRAINS SIMILAR	251	75	30	38	21	4	7
NOSE AND LESION STRAINS DISSIMILAR OR NO STRAIN ISOLATED FROM NOSE	77	72	14	28	51	0	7

CHARACTERISTICS OF STRAINS OF STAPH. AUREUS ISOLATED FROM LESIONS OF PATIENTS IN GENERAL PRACTICE.

TABLE 62.

C. INVESTIGATION OF STAPHYLOCOCCAL INFECTIONS IN NURSES

Staphylococcal infection among nurses and doctors in hospital is an important problem. The incidence of these lesions would appear to be quite high and it is probable that only a fraction are noted. Much working time is lost because of these lesions, especially by those nurses who have recurrent lesions. The most important feature of staphylococcal lesions among staff however, is that they are a source of infection to other persons, and outbreaks of hospital staphylococcal infections have recently been attributed directly to a nurse or doctor appearing on duty with a staphylococcal lesion. (McDonald and Timbury 1957)

It may be that these staphylococcal lesions are merely another sign of cross-infection occurring in hospital and that their epidemiology is similar to that of operation wound infections. However, as the nurses in hospital are known to be heavily colonised with nasal staphylococci it seemed worth while to investigate this problem and measure the true incidence of lesions, and study their epidemiology.

This investigation was carried out among the nursing staff of the Royal Infirmary, Edinburgh. The extent of nasal carriage of Staph. aureus among these nurses was already known in some detail as a result of the observations described above.

All nurses reporting with lesions were examined by Dr. Verney. Swabs from the lesions and nose swabs were sent for bacteriological examination. The strains of Staph. aureus were isolated and examined by the methods already described.

2: Incidence and nature of Staphylococcal infections among nurses.

The nursing staff of this hospital comprises approximately 700 nurses and sisters. From this number 200 persons, with a total of 330 lesions were examined during the two years from October 1956. This represents about 130 infections a year in 200 nurses and sisters, or a rate of about 14 per cent per annum. However, these lesions seen represent only a fraction of the total incidence since it was known that many lesions were not reported either to the ward sisters, nor to the medical authorities. It is probable that the true incidence of infection was in excess of 20 per cent per annum.

These lesions occurred among nurses at all stages in their training. A relatively large number of infections occurred amongst 1st year nurses. This supports the clinical impression that the young nurse is more susceptible than those who have been in hospital some time and may be correlated with the increased exposure of these girls to Staph. aureus on entering hospital and the increase in their nasal carrier rate of the organism. Thus, 29 per cent of all the lesions examined occurred among 1st year nurses who comprised 21 per cent of the nursing staff at risk. The proportion of lesions occurring in the other groups of nurses and sisters corresponded to their relative numbers of the total staff. (Table 63)

The lesions occurred with the same frequency in nurses engaged on the surgical medical wards of the hospital and in the maternity pavilion. Thus, 55 per cent of the lesions occurred in surgical ward nurses who comprise 51 per cent of the nursing staff of the hospital. Similarly, 31 per cent of the lesions occurred in medical ward staff who comprise 34 per cent of the staff, and 14 per cent in maternity staff who account for 15 per cent of the total staff. (Table 64)

Of the 200 nurses with lesions who were examined, 90 per cent were persistent nasal carriers. This is significantly higher than the carrier rate for the nursing staff as a whole which was 52 per cent at this time. In 70 per cent of the nurses with lesions the strain of Staph. aureus in the nose was identical in bacteriophage type with that isolated from the lesion. (Table 65)

Staphylococcal infection occurring among the nurses were similar in many respects to those occurring among the persons who were examined in general practice. In both groups of persons the majority of those suffering from lesions were nasal carriers of Staph. aureus and in most cases the nasal strain was identical to that in the lesion. This would indicate that the source of many of the infections in nurses was endogenous. Also in favour of this hypothesis was the nature of the lesions. Most were clinically similar to those found in general practice and the majority did not involve breaching of the skin or other body surfaces, as is the case with many instances of hospital cross-infection in patients. It is noteworthy that few infected wounds were present in the series of nurses' infections. (Table 66). The relatively high proportion of finger infections may be an instance of infections that were exogenous in source and analgous to the post-operative wound infections in patients, since the fingers are more readily traumatised in nurses during repeated washing and wetting of the hands during work.

The strains of Staph. aureus isolated from the nurses' infections, however, were the same as those isolated from the hospital environment, carriers, patients' lesions, nurses' lesions and dust. It is therefore impossible to be certain that infections did not result from one of these sources other than the nurses themselves /

themselves, or were due to contact with a reservoir in the hospital environment. It is certain that a number of cases do arise in this way. It is likely that the majority of infections among nurses were autogenous, but that a proportion are due to cross-infection or contact with another case.

YEAR OF TRAINING	NUMBER OF NURSES AT RISK	PROPORTION OF TOTAL STAFF %	NUMBER OF LESIONS	PROPORTION OF ALL LESIONS %
FIRST	156	21	98	29
SECOND	176	24	69	21
THIRD	154	21	64	20
FOURTH	99	13	40	12
STAFF NURSES	37	5	9	3
SISTERS	50	6	16	5
MATERNITY HOSPITAL STAFF	130	10	35	10

OCCURRENCE OF STAPHYLOCOCCAL LESIONS IN NURSES

AT VARIOUS STAGES IN THEIR TRAINING.

TABLE 63.

DUTY GROUP	NUMBER OF NURSES AT RISK	PERCENTAGE OF TOTAL OF NURSING STAFF	NUMBER WITH LESIONS	PERCENTAGE OF ALL LESIONS
SURGICAL WARD STAFF	450	51	165	55
MEDICAL WARD STAFF	300	34	103	31
MATERNITY WARD STAFF	130	15	46	14

OCCURRENCE OF STAPHYLOCOCCAL LESIONS IN NURSES

ENGAGED ON DUTY IN DIFFERENT PARTS OF THE HOSPITAL.

TABLE 64.

SOURCE OF STRAINS	NUMBER OF STRAINS	PERCENTAGE OF STRAINS WITH BACTERIOPHAGE PATTERNS OF GROUP					ANTIBIOTIC SUSCEPTIBILITY PATTERN			
		I	II	III	Unclassified	NT*	PR:SS CS:TS	PR:SR CS:TS	PR:SR CR:TS	PR:SR CR:TR
NURSES LESIONS	256	47	8	36	0	9	96	60	12	44
Nurses NOSES	230	38	5	42	5	15	97	53	16	38

* = N.T.

CHARACTERS OF STRAINS OF STAPH. AUREUS ISOLATED FROM LESIONS AND ANTERIOR NARES OF NURSES WITH STAPHYLOCOCCAL LESIONS.

TABLE 65.

Type of Lesion.	Percentage of cases of Staphylococcal Infection seen in		
	General Practice	Hospital Patients	Nurses in Hospital
Boils and Septic Spots	68	6	35
Styes	19	2	19
Paronychia and Septic fingers	8	7	28
Carbuncle	1	3	1.5
Sycosis barbae and Impetigo	1	1	< 1
Axillary abscess	1	-	7
Septic feet or toes	2	1	2
Infected wounds	0	62	3
Breast abscess	0	10	1
Respiratory tract infections	0	4	2
Urinary Tract infect-:ions	0	4	0

Clinical Types of Staphylococcal Infections Found In Hospital and General Practice

TABLE 66

Colonisation of the Anterior nares of Nurses with Staphylococcus aureus during training.

A group of 50 nurses joining the Pre-training School (PTS) of the Edinburgh Royal Infirmary were observed at intervals during the greater part of their 12 weeks training, and before taking up whole time duty in the hospital wards.

Four samplings of their nares were made at fairly regular intervals, and during the time covered by the three initial samplings these nurses were in class-rooms only, but before the fourth examination they had done 10 days part-time duty on the wards.

Of the 49 nurses regularly examined 8 had worked previously in a hospital environment. Only two of these nurses, however, were nasal carriers, and in one the strain of Staph. aureus was resistant to several antibiotics and had a phage pattern of Group I.

The proportion of these nurses yielding significant numbers of Staph. aureus from the anterior nares was approximately the same as in the general population (Page 95) and the majority were susceptible to penicillin, and with the exception of the strain mentioned above from the nurse who had worked in another hospital, were all sensitive to the other antibiotics tested. There was a relatively large proportion of strains with phage patterns of Group III. (Table 67)

After /

After 6 weeks at PTS two of the nurses not previously yielding Staph. aureus from the anterior nares now did so, and appeared to be colonised with strains of Group III patterns which were penicillin-resistant. Two other nurses who were colonised at the start of the investigation appeared to become recolonised with penicillin-resistant Group III strains. During the first few weeks, therefore, there had been a moderate degree of replacement and colonisation with strains of Staph. aureus, penicillin-resistant and of Group III phage patterns.

After part-time duty in the wards the change in the nasal flora was more marked. A much increased number of the nurses now yielded Staph. aureus, and in those newly colonised the strains were predominantly Penicillin-resistant and of the hospital phage types.

There was thus evidence of a rapid change in the quantity and quality of the strains of Staph. aureus colonising the anterior nares of these nurses during training, so that by the time they were ready for full-time duty on the wards their nasal flora resembles the remainder of the staff.

The same sort of evidence was forthcoming from the follow-up examinations carried out on nurses starting work in Leith Hospital during the investigation into infections previously reported (Section, p 197). During the period of observation December to May, 48 nurses started work in the hospital. A few had worked in other hospitals and were already carriers of penicillin-resistant Staph. aureus. The majority of the others were colonised with penicillin-susceptible strains.

Eighteen /

Eighteen of the 48 nurses were colonised with Staph. aureus on starting work in the hospital. Thirteen of these strains were penicillin-susceptible (Table 68) and 7 were replaced by penicillin-resistant strains, all but one (Pattern 52) being of different phage pattern to the original strain. Further, 3 penicillin-resistant strains were replaced by antibiotic-resistant strains of hospital pattern. Two penicillin-resistant strains remained with the same pattern. Three nurses, not previously nasal carriers had become so by the end of the period of observation. Thus, there were 21 carriers, of whom 15 were colonised by penicillin-resistant Staph. aureus .

Thus, while there was not a dramatic increase in the number of those colonised, there was a marked change in the antibiotic-resistance of the carrier strains showing that the newly-arrived nurses acquired the hospital strains of staphylococci.

Percentage Yielding Significant Numbers of Staph. aureus	Number of Strains	Percentage of strains with Bacteriophage Patterns of				Penicillin Resistant	Examination
		Group I	Group II	Group III	Not typable		
38	19	21	21	37	21	28	First Attending P.T.S.
41	20	15	15	45	25	40	2 weeks later
43	21	20	24	51	5	38	4 weeks later
67	30	43	14	33	10	67	Attendance in wards 8 weeks later.

Characters of Strains of Staph. aureus isolated from 49 Trainee Nurses on Four Occasions during Training.

TABLE 67

Number of Strains	Percentage of strains with Phage Pattern of Group				Percentage of Strains Penicillin Resistant	
	I	II	III	Not Typable		
18	17	39	22	22	28	On Starting in Hospital
21	14	19	43	24	72	At end of observations

Characters of Strains of Staph. aureus isolated from 48 nurses in Leith Hospital,

(a) On starting work ; (b) At the end of the Period of Observation.

TABLE 68

D. AN INVESTIGATION OF STAPHYLOCOCCAL INFECTIONS IN MATERNITY WARDS

Staphylococci are noted for the number of infections they produce in maternity units of hospitals. The number of these infections contracted in hospital is probably much greater than the figures usually quoted, for a great many develop after return to home and become the problem of the general practitioner. The same is true of many of the breast abscesses that develop in mothers after discharge from hospital. Staphylococcal infections in babies are serious in a minority of cases and a fatal outcome is not infrequently reported.

A study of the distribution of Staphylococcus aureus in maternity units was thus undertaken, first in the Simpson Memorial Maternity Pavilion during 1957-58, and secondly in the Eastern General Hospital Maternity Unit during 1958-59. This work was carried out with the co-operation of Mr. I.C.S. Knight and Dr. F.O. Forfar.

The Study of the Distribution of Staph. aureus in a Maternity Ward of the Simpson Memorial Maternity Pavilion.

Initial studies were made to determine the frequency and distribution of Staph. aureus in the ward and its environs. Mothers were examined for carriage of the organism on admission and discharge. The babies were examined for colonisation of their anterior nares, naso-pharynx, mouth, umbilicus and other skin areas on discharge, at 8 - 10 days after birth. All members of the ward staff, including doctors, nurses and ancillaries were examined for nasal carriage of Staph. aureus. In addition, the breast secretions of mothers examined for staphylococcus.

Further examination of the environment was carried out by direct sampling of fomites in the ward, and of air-borne dust at various sites.

2: The Isolation of Staph. aureus

Of 118 mothers examined on admission 41 (34 per cent) had moderately large or large numbers of Staph. aureus present in their anterior nares. (Table 69). Confirmatory serial sampling to establish the proportion of persistent carriers was not possible but on discharge 37 (31 per cent) had significant numbers of staphylococci in their nares ; of these, 7 were negative on admission and in a few the hospital organism was present. Thirty of the mothers were positive on admission and on discharge they were /

were still colonised by the same strain. The true nasal carrier rate was therefore probably nearer 25 per cent.

From a closer study of the antibiotic and bacteriophage susceptibility of the strains of Staph. aureus isolated from the mothers it was clear that there was no significant change in the nasal flora as a result of their short sojourn in hospital. (Table 70).

Among the 41 strains isolated from the mothers on admission there were 32 distinct phage patterns or 'types', of which 29 occurred only once, including one strain of 'type' 80, resistant to several antibiotics. Five strains were lysed by phage 79, three by 73, and four strains did not react to phages, either undiluted nor at RTD. Eighty per cent of the strains were susceptible to penicillin.

One hundred and ninety-five babies were examined from January to April inclusive. About 80 per cent of these infants had large numbers of Staph. aureus in their anterior nares or naso-pharynx on discharge 8-10 days after birth and in nearly all the numbers of staphylococci were sufficiently large to result in confluent growth on primary isolation. From this it can be surmised that the numbers of staphylococci obtainable from these sites exceeded that obtained from many staphylococcal lesions. (Table 71).

The number of staphylococci recovered from the anterior nares and naso-pharynx was approximately equal in 56 of the babies ; larger numbers were isolated from the naso-pharynx than nares in 78, and in 11 there were Staph. aureus present in the naso-pharynx but none in the anterior nares.

In 21 cases there were more Staph. aureus in the nose than in the naso-pharynx, and in 8 the organism was present in the nose without recovery from the naso-pharynx.

In the 155 cases where the staphylococcus was isolated from both sites it was identical in all but 5 (3 per cent).

Seven mothers on discharge and the same strain colonising their anterior nares as that colonising their baby. In two of these the strain had replaced the "natural" carrier strain of the mother. In one other case the mother was admitted already colonised with a 'type 80' so that the strain could have colonised the baby either from the environment or the mother.

The saliva of 91 babies was examined for Staph. aureus. Staph. aureus was not found in the saliva of adults (Table 41, Page 167) as it appears to be destroyed in the mouth and cannot compete with the normal oral flora. In this series the organism was isolated from 32 of 91 babies, but in only 10 (11 per cent) were the numbers large enough to be of any significance. (Table 72).

The breast secretions of 94 mothers were examined for Staph. aureus and in 26 significant numbers of the organism were recovered from one or other breast, and in 21 the strain was the same as that isolated from the nose or naso-pharynx of the baby, but in only 5 did the mother's baby have large numbers of Staph. aureus in its saliva. In 2 cases the organism isolated from the breast secretion corresponded with the baby's naso-pharyngeal strain where this differed from that of the anterior nasal strain. (Table 73).

Small numbers of Staph. aureus were isolated from other parts of the open surfaces of the babies (the umbilicus was not examined to any great extent in this series) but the numbers isolated were no greater than would be expected from contamination of these surfaces from other sources and reservoirs in the environment; it was therefore concluded that colonisation and growth of Staph. aureus had not occurred at these sites.

3: Nature of the Babies' strains

The strains of Staph. aureus isolated from the babies were entirely different in bacteriophage type and antibiotic susceptibility from those isolated from the mothers. (Table 70). The number of phage patterns was limited so that three patterns "80", 79" and 77" accounted for 90 per cent of the strains, and nearly all of them were resistant to penicillin.

These strains isolated from the nose and nasopharynx of the babies were found among the staff carriers and occurred in appreciable numbers in the environment.

The colonisation rate of the anterior nares and naso-pharynx of the babies remained high during January, February and March, but fell to 65 per cent in April. (Table 74). There was a marked change in the distribution of the predominant phage types, for in January the predominant colonising strain was 'type 80', which was present in 70 per cent of the babies, but in February this /

this strain was isolated from only 40 per cent of the babies examined, and in March from only 25 per cent. Strain '77', present in only small numbers in January increased steadily so that it was isolated from 62 per cent of babies in April. Strain 79 was more common in March than in any other month.

4: Staph. aureus in the nursing and medical staff.

The nurses, doctors, cleaners, ancillaries on the staff of the unit were examined at intervals for nasal carriage of Staph. aureus. (Table 74).

In January 18 of 32 members of staff and moderately large or large numbers of Staph. aureus in their anterior nares (56 per cent). Seventy-five per cent of the strains were penicillin-resistant and the majority had phage patterns of Group III. Three strains were of pattern 54/73 and 2 of "77".

In February the nasal carrier rate was 60 per cent, 80 per cent of the strains were penicillin-resistant. Four strains were of type "77" and 1 was of type "80". In March, the carrier rate was again 60 per cent, and 86 per cent were penicillin-resistant.

Several nurses examined yielded large numbers of Staph. aureus from their hands where these were impressed on the surface of agar plates, others only a few. One doctor had a few on his hands, another large numbers.

5: Staph. aureus in the environment

Staph. aureus was isolated from table tops, ward screens, cots, blankets, towels, face masks and various fomites in varying numbers. Swabs swept over various articles yielded significant numbers of Staph. aureus and this may be used as a comparative guide, for example 12 swabs swept over the babies changing room yielded 104 Staph. aureus, 12 swabs swept over the nursery

yielded 11 colonies.

Milk-agar and blood-agar plates flapped over the surface of soft articles recovered Staph. aureus which could be counted if the other flora were selectively checked.

Air-borne dust was sampled as previously described. The number of Staph. aureus contaminated particles varied considerably from one part of the unit to another but was undoubtedly consistently higher in the changing room where frequently more than 100 Staph. aureus particles per 100 cubic feet were recovered. (Table 75A)

	Number of Mothers' Nose Swabs	
Number	Positive for *Staph. aureus* on Admission.	Negative for *Staph. aureus* on Admission
	41	77
Positive on Discharge		
37	30	7
Negative on Discharge		
81	11	70

Recovery of Staph. aureus from Mothers on Admission and Discharge from Maternity Hospital.

TABLE 69.

Source of Strains	Number of Strains	Percentage of Strains with Bacteriophage Susceptibility Pattern of: Group I	Group II	Group III	Not Typable	Percentage Penicillin resistant
Mothers on admission	41	30	20	41	9	17
Mothers on discharge	37	27	22	46	5	19
Babies Nares	137	38	3	55	4	97
Hospital Staff Nares	52	16	10	68	6	86

Characters of strains isolated

in the Simpson Maternity Pavilion.

TABLE 70.

Number of Babies Examined	Percentage of babies in whom Staph. aureus was Isolated in large numbers from :			Not isolated From Nose or Naso-Pharynx
	Nose and Naso-pharynx	Nose Only	Naso-pharynx Only	
195	72	5	4	19

Isolation of Staph. aureus from Anterior Nares and Naso-pharynx of Babies.

TABLE 71.

Number of Babies' Mouths Examined	Percentage of Babies in whom Staph. aureus isolated in large Numbers	in small Numbers
91	11	24

Isolation of Staph. aureus from Babies' Mouths

TABLE 72.

Isolation of Staph. aureus from Mothers' Breast Secretions.

TABLE 73

Number of Mothers' Breasts Examined	Number from whom Staph. aureus isolated in significant numbers from either breast	Number in whom Staphylococcus identical to Baby Carrier strain.
94	26	21

		Jan.	Feb.	March	April	May	June
Number of babies		39	42	38	36	24	31
Percentage colonised with Staph. aureus		87	71	81	64	25	61
Number of strains examined		34	30	31	23	6	19
Percentage of 'Type'	80	70	40	26	30	16	21
	79	9	3	35	0	0	5
	77	12	47	39	61	50	21
	Others	9	10	0	9	34	53
Number of Staff examined		32	25	23		23	21
Percentage with Ant.Nares colonised with Staph. aureus		56	60	61		9	14
Percentage colonised with 'Type'							
	80	0	7	7		0	0
	77	89	60	65		50	33
	Others	11	33	28		50	67
Percentage of babies strains Penicillin resistant		100	97	97	100	100	100
Carrier strains Penicillin resistant		78	80	86		100	100

Isolation of Staph. aureus from babies and carriers among Staff in a maternity unit (S.M.M.P.).

TABLE 74.

SITE	Number of observations	Average number of colonies of Staph.aureus recovered from: 12 swabs	Impression plate	Flap Plate
Woolen blankets, in use	8	-	-	16
Laundered woolen blanket	2	-	-	0
Cotton blanket, in use	4	-	-	15
Unused cot	6	-	-	0
Used cot	6	-	-	2
Table tops	4	-	-	6
Personal towels	12	-	50	20
Bed cover	4	-	-	2
Walls	5	11	-	-
Babies'changing room towel rack	11	104	-	-
Babies' towels in rack	12			
Fomites	6	7	-	-
Hands of Staff	14	-	245	-

Recovery of Staph. aureus from the environment in a maternity unit (S.M.M.P.)

TABLE 75A.

SITE	Number of Examinations	Recovered by Sieve-Sampler			Recovered on Settling Plates		
		Staph. aureus per 100 cub. ft.	Total bacteria per 100 cub. ft.	Staph.aureus as % of total bacteria	Staph.aureus settling per ft./hr.	Total bacteria settling per ft./hr.	Staph.aureus as % of total bacteria
Nursery	8	12	1880	0.64	1.5	76	2.0
Main Ward	4	20	2350	0.85	1.0	148	0.67
Small Ward	4	16	2600	0.62	2.0	123	1.62
Isolation Ward	3	4	2080	0.19	1.2	86	1.4
Corridor	4	14	2700	0.52	2.8	138	2.05
Duty Room	4	6	1900	0.32	2.1	104	2.01
Babies Changing Room	8	90	2700	3.35	5.8	180	3.24

Results of examination of air-borne and settling dust in maternity unit (S.M.M.P.).

TABLE 75.

II. 1: Distribution of Staph. aureus in the Eastern General Hospital Maternity Unit.

The investigation carried out in this unit differed in certain respects from that in the Simpson Memorial Maternity Pavilion but it was hoped that the information obtained would be complementary.

Only a proportion of the babies born were examined, usually the first born on each day during the period of the investigation. More extensive information about colonisation of the babies was sought by swabbing on the 2nd, 4th and 8th days after birth, and the umbilicus was examined along with the anterior nares and nasopharynx. In addition, infections occurring among the babies and mothers in the unit were examined for Staph. aureus. Other examinations included swabbing of the noses of the mothers on admission, frequent examination of the ward staff for nasal carriage and the presence of staphylococci on their hands, and surveys of the environment for Staph. aureus

2: Nature of the carrier strains isolated from members of staff.

The carrier rate among 59 nurses, doctors and ancillary staff was 44 per cent. The majority of the strains were penicillin-resistant and had phage patterns of Group III. (Table 76).

3: Nature of the strains of Staph. aureus isolated from the babies

The umbilicus was colonised at a much more rapid rate than the upper /

upper respiratory tract in the babies examined. (Table 77). Only a small proportion (8 per cent) of the babies were colonised by Staph. aureus in the anterior nares or naso-pharynx by the 2nd day after birth, but 15 per cent were heavily colonised at the umbilicus by this time. Nearly half the babies had the organism in the upper respiratory tract in large numbers by the 4th day when 90 per cent were colonised at the umbilicus. By the 8th day 70 per cent of the nares and 14 per cent of the naso-pharynges were colonised. At the first follow-up, 18 days after birth, nasal carriage had risen to 81 per cent, but contamination of the umbilicus was reduced to 55 per cent (Table 77).

The nose, naso-pharynx and umbilicus were colonised in 66 per cent of the babies examined, and in two -thirds of these the same strain was isolated from all three sites.

The great majority of the strains colonising the babies had Group III phage patterns, and over 90 per cent were resistant to penicillin, and over 80 per cent were resistant to streptomycin. Smaller proportions were resistant to the other antibiotics including erythromycin. A single pattern - 47/77 accounted for 69 per cent of the strains isolated from the upper respiratory tract of the babies. The antibiotic susceptibility pattern of this strain varied considerably; in the majority of isolates it was resistant to penicillin and streptomycin and sensitive to the other antibiotics, but on 30 per cent of occasions it was resistant to tetracyclines ; in 20 per cen to chloramphenicol, and in 11 per cent to erythromycin. It was the only erythromycin resistant strain isolated in the hospital.

4: Mothers' strains

The anterior nares of 24 per cent of the mothers were colonised with Staph. aureus. Two-thirds of these strains were susceptible to penicillin and the phage patterns were many and varied, and did not correspond to the strains recovered from the babies. (Table 76)

5: Nature of the strains isolated from the babies' lesions

The strains of Staph. aureus isolated from 132 episodes of infection in the babies were examined and found to be similar in phage pattern and antibiotic susceptibility to these carried by the nursing staff and the babies themselves. (Table 76).

The lesions were almost all of a minor nature comprising pustules and septic spots (25 per cent), infections of the eye (52 per cent) and fingers (11 per cent). A small number were boils and abscesses (5 per cent).

The same strain, of phage pattern 47/77 was isolated from 84 (64 per cent) of these lesions.

6: Strains of staphylococci from the infections

All the infections examined, occurring in the babies, mothers or other persons in the hospital during this time were found to be due to the strains of Staph. aureus described above.

7: Staph. aureus in the environment

In the environment Staph. aureus was isolated from the dust adhering /

	Percentage colonised in anterior nares	Number of strains	Percentage of strains with bacteriophage pattern or:				Percentage of strains Penicillin resistant
			Group I	Group II	Group III	Not typable	
Mothers	24	17	18	23	36	23	33
Babies	70	73	10	4	86	0	93
Nurses and other staff	44	26	15	15	66	4	85
Babies lesions	-	132	9	18	71	2	96

Characters of carrier strains of Staph. aureus isolated from mothers, babies and members of staff in the E.G.H. Maternity Unit.

TABLE 76.

Day after Birth of Sampling	Percentage of Babies colonised By Staph. aureus in		
	Anterior nares	Naso-pharynx	Umbilicus
2	8	7	75
4	43	46	90
8	70	74	90
18	81	77	55

TABLE 77

RECOVERY OF STAPH. AUREUS FROM BABIES DURING THREE WEEKS AFTER THEIR BIRTH.

adhering to the walls, air-borne dust, fomites, bedding and laundry. The largest numbers were recovered from the nurseries and the changing rooms, the smallest numbers were found in the premature ward where the temperature and humidity were high (70 per cent)

8: Distribution of Staph. aureus.

A striking fact was the predominance of one strain throughout the maternity unit (and possibly more widely through the hospital). This strain, of phage pattern 47/53/54/73/75/76/77, was similar, if not identical to the strain "77", isolated in the Royal Infirmary Maternity Unit, and elsewhere in this geographical area. Our evidence shows it to have been present for a long time in this area, appearing during the investigation in Leith Hospital in 1953-54, when it was resistant to penicillin, and rarely resistant to other antibiotics. In more recent investigations the same organism has been more often resistant to many antibiotics and in the present situation it was notable for being resistant to penicillin, streptomycin, chloramphenicol, the tetracyclines and in about 20 per cent of isolations to erythromycin. The second most frequently isolated strain was type 80 (pattern 42B/52/52A/73/80 and variations) usually resistant to penicillin and streptomycin and more rarely to other antibiotics but never erythromycin. Relatively few other strains of Staph. aureus were encountered either from the babies, staff or environment.

environment. One strain, Group II colonised a few babies within a short time of one another and was also isolated from several staff carriers .

Thus the majority of the babies and members of staff were colonised with the same strains of Staph. aureus, and with much the same frequency for each strain. The air-borne dust contained appreciable numbers of the same organisms as did the surfaces of fomites and bedding.

It was obvious that the umbilicus of the babies was colonised heavily at a very early stage and in most cases the nares and naso-pharynx were colonised with the same strain a few days later. However it is worth noting that in some babies the strain of Staph. aureus colonising the upper respiratory tract was different from that colonising the umbilicus. This indicates that the sources of contamination of all sites on the body was probably exogenous and that the nares and naso-pharynx were not colonised from the umbilicus. The conditions for establishment of the contaminating staphylococci in the nares and naso-pharynx must be a little more difficult.

A limited number of follow-up studies showed that the Staph. aureus was lost from the umbilicus soon after the cord separated, but persisted in the nares and naso-pharynx as long as the follow-up extended.

There /

There can be little doubt that under these conditions the babies were assaulted from the first minutes of life with relatively large numbers of Staph. aureus which were able to successfully colonise certain sites on their bodies. These organisms must reach these sites by the following routes.

(a) Inhalation of Staph. aureus infected particles in air-borne dust.

The degree of contamination of the airborne dust in this unit ensured that each baby inhaled several dozen such particles each day so that the chances of contamination of the nasal passages by this means alone must be 100 per cent.

(b) Direct touch from attendants who may be carriers or transfer staphylococci from other carriers or reservoirs in the environment.

(c) Contamination from reservoirs in the environment, particularly towels and clothes and bedding.

(d) Indirect or direct contact with other babies who are already colonised.

The Epidemiology of Staphylococcal infections

The results of the experiments reported have shown clearly that Staphylococcus aureus was widely distributed through the environment but that the sources can be distinguished as the sites at which the organism was multiplying and are almost always limited to carriers and persons suffering from lesions.

Staph. aureus was isolated from a large proportion of the healthy individuals in all sections of the community that were examined, but not all these persons were carriers. The true carriers were those harbouring the same strain for lengthy periods, at least many months and probably years in most cases. In the majority the anterior nares was the primary site of colonisation, though in a small number of persons sampled a significant number were colonised at other sites. Nasal carriers were classified as persistent when a nose swab frequently sampled large numbers of staphylococci, and intermittent when the swabs yielded staphylococci irregularly. Both these groups of persons disseminated large numbers of Staph. aureus of the same type as found in their nares to their own skin, handkerchief and clothing, and thence to the environment or direct to other persons.

In many other persons examined nose swabs occasionally yielded smaller numbers of coagulase positive staphylococci, often of mixed phage types. It seems most likely that these /

these persons were contaminated by inhaling the organism in air-borne dust since the number of such persons corresponded to the number of Staph. aureus recovered from the air-borne dust of their environment. The frequency of these contaminated persons can therefore be used as an indicator of the degree of contamination of an environment.

Staphylococcal lesions were examined in patients who had contracted infection in hospital, and at home among patients who had had no contact with hospital. The home lesions were predominantly superficial in character and were not associated with any apparent breach of the body surfaces at the site of the lesion which would predispose to establishment of the organism.

In hospital, the lesions were in two groups, one occurring among hospital personnel and the other among hospital patients. The lesions in hospital staff were clinically similar to those occurring in patients at home. Infections in hospital patients, which included a number of deeper and more serious lesions, were different because damage to the tissue predisposing to the establishment of infection was present.

The strains of Staph. aureus isolated from the home environment were extremely diverse in their phage 'types'. Group II strains, rarely isolated in the hospitals were almost as numerous as strains of the other Groups. Only 20 per cent were resistant to penicillin and very few to other antibiotics. The hospital strains comprised relatively /

relatively few 'types', mainly in Phage Groups I and III, and almost all strains were resistant to antibiotics.

Between these two environments the most striking differences were observed in the association of nasal carriage with staph.-ylococcal lesions. Ninety per cent of the patients with lesions contracted at home were persistent carriers, and in the majority the strain in the nose was identical with that isolated from the lesion. A high proportion of the hospital nurses with staphylococcal infections were persistent nasal carriers and in the majority there was identity between the carrier and lesion strains. The results were entirely different in the hospital patients, for excluding new-born babies, only 20 per cent of patients with staphylococcal infections contracted in hospital were persistent nasal carriers, and there was little or no correspondence between carrier strain and lesion strain in these cases. The strains isolated from these hospital patient infections were the same as those isolated from hospital personnel carriers and the hospital environment.

It must be concluded therefore that most staphylococcal infections in hospital patients were the result of cross-infection from hospital staff carriers, or lesions among staff and patients ; on the other hand the majority of staphylococcal lesions in patients at home and among hospital personnel were autogenous.

In all the cases of staphylococcal infection described, both /

both in and out of hospital, the nasal carrier has proved to be of epidemiological importance as one of the major sources of the causative organism. The difference in the epidemiology of these cases of infection lies essentially in the routes of spread of the staphylococcus.

The sources of the Staph. aureus, namely the carriers and cases of infection disseminate the organism to themselves, to the dust of their environment, to fomites, including bedding and clothes, and directly to other persons. The control of staphylococcal infections must involve the interruption of these various routes of spread, and many methods of preventing cross-infection in hospital have been directed to this end. The success of these methods in preventing staphylococcal infections, has been variable which in itself may be a reflection of the relative importance of the different routes of spread in each situation, or the efficiency with which the measures have been put into practice and the enthusiasm with which the experiments were followed. However any method which is designed merely to inferfere with the routes of spread of the staphylococci, or reduce the number of organisms in the environment such as dis-infection, can be only partially successful in reducing infections since the sources remain free to recontaminate the environment.

Suppression of Staph. aureus at source should eliminate

or /

or reduce contamination of the environment, and be more effective than multiple methods designed to interrupt intermediate spread of the organism. Contamination of the environment should cease and the number of staphylococci already in the environment should decrease as the organism dies or is removed. This process of death and removal could be accelerated by cleaning and disinfection.

Individuals with lesions are obvious and should be isolated and treated so that the spread of their staphylococci is limited. Carriers on the other hand are much more numerous, less obvious and their movement cannot be effectively restricted. They produce large though variable numbers of Staph. aureus and are a more continuous and dangerous source of the organism. If carriers are important in the spread of staphylococcal infections the suppression of the staphylococcus at the primary site of growth should reduce the contamination of the carrier and his environment and control the spread of the organism. As a result the number of staphylococcal infections, both autogenous and as a result of cross-infection should be reduced.

Staph. aureus in the nose appears to be readily accessible to the action of specific antibacterial agents, so that suppression, if not eradication should be achieved at this site of growth by the use of the appropriate agents. The possibility of effecting such suppression and control of nasal Staph. aureus and its efficiency in reducing spread of the organism and the production of infections has been measured in four ways to be described.

1: /

1: The first of these was the conversion of nasal carriers to free them temporarily of Staph. aureus. If this organism cannot be recovered from the nose on sampling it would be unlikely that significant numbers would be disseminated from the nares.

2: The efficiency of suppression of the staphylococcus in the nares was assessed by measuring quantitatively, the dissemination of Staph. aureus from the skin, upper respiratory tract, clothes and handkerchief of selected carriers both before and after suppression of the nasal organism. The result indicated that a treated carrier disseminates less than 10 per cent of Staph. aureus to the environment liberated by an untreated carrier.

3: The effect of controlling nasal staphylococci on the incidence of hospital staphylococcal infection has been observed. As a result of reducing the number of disseminating carriers in the hospital staff to a relatively low level, the incidence of cross-infection fell, and remained low as long as the carrier rate was low.

The same effect was observed on the rate and extent of colonisation by Staph. aureus of babies in a hospital maternity unit after the staff nasal carriers had been controlled for a short period.

4: The incidence of autogenous infection among patients in general practice was examined before and after controlling Staph. aureus /

4: continued

Staph. aureus in their nares. The result of this form of prophylactic treatment was a marked fall in the incidence of staphylococcal infection among treated individuals.

An Investigation of the Use of Antibacterial Agents to Control the Growth of Staphylococcus aureus in the Anterior Nares.

It was observed that during systemic therapy with antibiotics the staphylococcus, and other micro-organisms, frequently disappeared from the anterior nares of carriers (Gould and Cruickshanks). This was presumably due to the inhibitory effect of circulating antibiotic at the site of growth of the organism. However this effect was by no means constant, so that the large amounts of antibiotic given parenterally were not fully effective in suppressing locally growing antibiotic susceptible staphylococci in the anterior nares.

Staph. aureus. in the anterior nares would appear to be readily accessible to the action of specific antibacterial agents applied to the surface of the skin of the nares since it seemed probable that this method brings a higher concentration of antibacterial agent to the actual site of growth.

Thus an antibacterial drug could be applied in aqueous solution in the form of instilled drops; or as an atomised spray, as powder or as snuff, or carried by a vehicle in an ointment. Owing to the nature of the area to be treated in nasal carriers a spray or snuff was likely to be wasteful and will lead inevitably to much contamination of the environment by the antibacterial agent. Also aqueous solutions were difficult to apply and their effect would be intermittent unless very frequently applied. More satisfactory was an ointment, or cream base to carry the active agent /

agent as this preparation adhered to the surface of the skin and acted as a reservoir of inhibitory material which was continuously released to the underlying skin and its glands in which the staphylococcus was growing. This allowed intermittent application of the antibacterial ointment or cream and a more continuous effect.

Since antibiotics are weight for weight the most effective antibacterial agents, as well as having a high chemotherapeutic index (Ehrlich) initial observations were carried out incorporating these agents in suitable ointment and cream vehicles.

2: Choice of Vehicle for the antibacterial agent.

The object was to bring an active antistaphylococcal agent into intimate contact with the skin of the nares. Rapid release of the agent from the preparation applied to the nostrils was desirable so that effective concentrations were available to inhibit the staphylococci.

Most of the active agents to be considered were water soluble and therefore oily or paraffin-type bases were less efficient than water-in-oil emulsions in releasing antibiotic or antibacterial agents to the surrounds when tested in vitro.

Also the water-in-oil emulsions were aesthetically preferable and seemed to be better tolerated cosmetically by persons repeatedly administering them to the anterior nares.

3: Preparation of Nasal antibacterial cream.

Lanette wax (SXX) was melted in a water bath and three volumes of hot water added and rapidly mixed to form a stiff cream (Emulsion of water in oil). This material was opaque and white in colour and could be stored indefinitely.

The antibiotic or antibacterial agent was freshly prepared in aqueous solution and added to the cream base to give the required weight/volume concentration of active agent. The mixture was vigorously stirred to give a smooth emulsion of the consistency of whipped cream. The final concentration and uniform dispersion of the antibacterial agent was checked by assaying different batches of the mix.

These preparations were bland; they did not smell nor show in the nostrils if excess was removed; they were non-greasy and most carriers were not aware of the presence of the material after the first few applications.

4: Use of antibacterial creams to suppress nasal Staph. aureus.

Persistent nasal carriers who had been observed for at least 2 months were chosen for these experiments. As far as could be determined they were carriers of only one phage type of Staph. aureus.

The antibacterial cream was applied several times each day to the skin of the nostrils with an applicator, and rubbed in with the tip /

tip of the finger. The carriers were asked to apply the cream 6 times a day, applying an amount, about the size of a grape-stone (25mgm) to each nostril. Control groups of persistent carriers receiving no antibacterial cream and cream containing no active agent were examined at the same time.

Nasal swabs from each of the carriers were examined weekly, or more frequently, for Staph. aureus over a period of at least 20 weeks. The strains isolated were investigated in detail and compared with the strains isolated before treatment started.

5: Experiment to find the minimum effective concentration of antibiotic.

Preliminary experiments with creams containing penicillin showed that a concentration below 1000 units per gram was usually ineffective in producing nose swab cultures free of Staph. aureus, even during the period of application of the cream. Concentrations up to 10,000 units per gram were increasingly effective, presumably because the higher concentrations were required to overcome loss of an antibiotic by ansorption and dispersal, and leave sufficient available to kill the organisms on the surface and penetrate into the glands where they were growing. Creams containing 100,000 units per gram were not appreciably more effective in clearing staphylococci from the surface of the nares than those containing 10,000 units per gram. (Table 79).

Similarly /

Similarly 0.1 per cent chloramphenicol, streptomycin, chlortetracycline, oxytetracycline and erythromycin in creams were much less effective than 1 per cent concentrations.

Since it has been reported that the development of resistance is more likely after exposure to high concentrations of antibiotic when sterilisation is incomplete (Gould, Bowie and Cameron, 1953) and since resistance is more likely to appear after prolonged treatment with antibiotic, it was decided to make most of the tests with antibacterial creams containing 1 per cent antibiotic, and to limit the initial period of application to 14 days.

6: The effect of antibiotic containing cream on antibiotic-sensitive staphylococci in the nares.

The carriers examined in this experiment were all colonised with penicillin-susceptible strains of Staph. aureus which would therefore be susceptible to the other antibiotic agents.

In all the nasal carriers treated with antibiotic cream there was a marked fall in the number of colonies of staphylococci isolated from nose-swab cultures made during the time the antibiotic was being applied.

One week after the start of treatment no colonies of Staph. aureus were isolated from 96 out of 124 carriers, while 117 of 120 controls were still strongly positive.

Oxytetracycline was most effective as Staph. aureus was absent from nose swab cultures taken from all the carriers who had had one week's treatment with this antibiotic; similarly, chlortetracycline cleared 86 per cent, penicillin 77 per cent, streptomycin 71 per cent and chloramphenicol 67 per cent.

Sixty-nine /

Sixty-nine per cent of all the treated carriers gave nose swabs culturally negative for Staph. aureus for 2 weeks or more after withdrawal of the antibiotic. Forty-nine per cent remained negative for at least a month, and 26 per cent for 20 weeks or more. (Table 80).

The period of clearing (suppression) of the staphylococcus in the anterior nares was approximately the same for all of the antibiotics tested. During the same period the rate of spontaneous change from the carrier to non-carrier state in the controls who were receiving no antibiotic in their nares was 1 per cent.

7: Suppression of antibiotic-resistant strains of Staphylococcus aureus in the nose

Eight carriers of penicillin-resistant penicillinase producing strains of Staph. aureus were treated with nasal cream containing 100,000 units of penicillin per gram. In four of these persons the organism disappeared from nose swab cultures for periods varying from 2 - 6 weeks ; in a fifth there was apparent replacement with a strain of different phage type which was penicillin-sensitive, and in the remaining three the original carrier strain persisted (related to the amount of penicillinase produced by individual strains.)

8: Observed changes in antibiotic susceptibility of the strains of staphylococci in the noses of the treated carriers.

From /

Antibiotic	Concentration of Antibiotic in cream	Number of carriers treated	Number with nose swabs negative for Staph. aureus	
			During Treatment	For 2 weeks after Treatment
PENICILLIN	100 units/Gm	6	0	0
	500 "	5	1	0
	1,000 "	6	2	1
	2,000 "	3	1	1
	5,000 "	4	3	2
	10,000 "	6	5	4
	100,000 "	5	4	4
STREPTOMYCIN	100 µg/Gm	4	1	0
	1,000 "	3	2	0
	10,000 "	3	3	2
CHLOROMYCETIN	100	3	0	0
	1,000	3	1	1
	10,000	2	2	2
TERRAMYCIN	100	4	0	0
	1,000	6	3	1
	10,000	6	6	4

Effect of different concentrations of antibiotic on nasal carriage of antibiotic-susceptible Staph. aureus

TABLE 79.

Antibiotic Cream Administered 7 - 14 days.	Number receiving antibiotic	Number in whom nasal swab negative for Staph. pyogenes					Number in whom:		
		After one weeks administration of antibiotic.	Weeks after withdrawal of antibiotic.				Original strain persisted or returned after temporary absence	Replacement strain temporarily present	Replacement strain persisted.
			1	2	4	20 or more			
Penicillin	44	34	11	33	20	11	32	16	-
Streptomycin	21	15	6	15	12	6	15	5	0
Chloramphenicol	18	12	9	9	9	6	12	3	0
Chlortetracycline (aureomycin)	21	18	6	15	12	6	15	1	0
Oxytetracycline (terramycin)	20	20	7	13	8	4	16	3	0
TOTAL	124	99	39	85	61	33	90	28	1

Isolation of Staph. pyogenes from nasal swab cultures following local application of various antibiotics. Occurrence of replacement strains.

TABLE 80.

From 24 carriers who received antibiotic containing nasal cream, antibiotic-resistant strains of Staph. aureus were isolated, and these strains were either of bacteriophage 'type' different from the original carrier strain, or did not react with the bacteriophages. Two-thirds of the resistant strains which were susceptible to bacteriophage had patterns of Group III. (Table 81).

Replacement with these resistant strains of Staph. aureus was a temporary episode, except in one carrier receiving penicillin cream in whom the penicillin-resistant strain isolated after treatment persisted during the whole period of observation. In a further four carriers who received penicillin-cream there was replacement for a limited period, with a penicillin-susceptible strain of different phage-type. In the remaining carriers a strain of the same antibiotic sensitivity and phage type as the original carrier strain returned in cultures after a few weeks, and the resistant strain disappeared.

There are relevant objections to the topical exhibition of antibiotics used systemically to treat serious infections. These are particularly pertinent in hospital.

1: Since antibiotic resistance to many of the commonly used antibiotics is common among strains of Staph. aureus, and other species of micro-organisms likely to be encountered in /

Carrier Number	Phage type of original strain	Phage type of replacement strain	Antibiotic sensitivity original strain	Antibiotic sensitivity replacement strain
		Penicillin (units/ml.)		
6	47A/47C	52	0.03	3
11	3A/3B	76/77	0.03	1.5
16	3A	47A/54	0.04	3
25	47C	47C/54/75	0.06	1.2
39	3A	29/42E	0.03	0.03
44A	52/52A	52A	0.03	2
45	47+	47A	3	0.03
47	47A	51	0.06	0.03
48	51	3B	0.03	0.036
66	3A/51	7/47/54	0.03	3
74	3A/3C	7/47/54	0.06	3
119	52	7/47/54	0.03	1
122	6/47A/54	NT *	0.03	2.25
126	3C/51	6/47/53	0.03	2.25
157	6/7/53	NT	0.03	2
171	52/52A	7/47/53	0.03	3
523	44A/52	NT	0.03	0.03
		Streptomycin (µg/ml.)		
411	3B/55	6/7/47/53	0.5	20
415	NT	76/77	1.0	30
420	54	NT	1.0	25
422	3B/3C/51	NT	1.0	25
440	52	NT	0.5	15
		Chloromycetin (µg/ml.)		
521	NT	52A	1.5	15
529	52A	NT	3.0	20
530	6/7/53	52A	2.5	20
		Terramycin (µg/ml.)		
5	42E/47/47C	47/54	0.25	6
11	52A	NT	0.5	25
43	3A	47/47A	0.2	250
		Aureomycin (µg/ml.)		
602	3C/51	NT	0.25	250

* not typable.

Bacteriophage type and antibiotic sensitivity of original and replacement strains

TABLE 81.

in hospital, the antibacterial agents described for use in nasal creams in the last section cannot be expected to be generally effective for suppressing the staphylococcus in the nares of hospital personnel or patients. In vitro susceptibility tests would require to be done in individual cases to ensure the use of a specifically active agent. For this reason penicillin containing antistaphylococcal nasal creams are of little use in treating nasal carriage in hospital personnel.

2: Resistant strains of Staph. aureus might be induced, or selected in the flora of the carrier by the antibiotic. This we have shown to occur in our series of cases where penicillin-susceptible strains were apparently replaced by resistant strains of different type. Though in most cases the reverse process took place on removal of the antibiotic and the original sensitive strain returned to the nasal flora, this cannot be said to be a desirable phenomenon to encourage.

3: Individuals with antibiotic in their nares might be a more than usually favourable environment for colonisation by antibiotic-resistant strains which contaminate the surfaces of the nostrils by inhalation or transfer by touch. This is a greater likelihood in the hospital environment since sampling of air-borne dust has shown that there are large numbers of these resistant strains in hospital than elsewhere.

In these ways the number of persons harbouring antibiotic-resistant /

resistant Staph. aureus could be increased and the spread of resistant strains through the environment increased.

Other possible effects are :

4: The carrier may be immunologically sensitised to the antibiotic, thereby becoming embarrassed in the case of future systemic therapy being required.

5: The natural flora of the anterior nares could be grossly upset so that replacement with strains or species of micro-organism not normally present occurs. So far we have not observed this type of change but great care has been taken to limit the concentration of antibiotic used in the topical preparations and the length of application of the material, so that the chance of any such change becoming established is reduced.

6: The use of topical antibiotic will inevitably lead to contamination of the environment with the antibiotic. The amount will be small in comparison to the total amount of antibiotic which can reach the environment from other sources (see page 318), but this contamination would be expected to result in the emergence and further spread of antibiotic-resistant strains through the environment.

For these reasons it was thought desirable to investigate the use of alternative antibacterial agents, which whilst having a potent effect in suppressing nasal staphylococci would be without the /

the drawbacks already referred to. A number of antibacterial substances were used in experiments similar to these described for measuring the effect of the antibiotics. A comparison of their efficiency in converting nasal carriers of Staph. aureus was made for the following substances,

(a) antibiotics not normally used for systemic therapy such as tyrothricin, neomycin, bacitracin and polymyxin B.

(b) antibacterial sunstances designed primarily for external application such as the propamidines, di-guanides and furazones.

Naturally occurring strains of Staph. aureus resistant to any of these agents were not numerous but should they become so the consequences would not be so difficult to content with as the emergence of strains resistant to antibiotics such as erythromycin. Similarly, the development of immunological hypersensitivity to the antibacterial agents and lesser used antibiotics would not be so serious from the long term point of view.

9: Comparison of the antistaphylococcal effect of various antibacterial agents when incorporated in nasal creams

Groups of persistent nasal carriers were treated with nasal creams containing the antibacterial agents listed in Table 82. Nasal swabs sampled these carriers at frequent intervals during and after the use of the creams for a standard period of 10 days.

The results (Table 82) indicate that these lesser used antibiotics and antibacterial agents were less potent in suppressing Staph. aureus /

ANTIBACTERIAL *	NUMBER OF CARRIERS TREATED	WEEKS AFTER TREATMENT NOSE SWABS NEGATIVE FOR S.AUREUS				DURING PERIOD OF TREATMENT
		More than 8	4-8	1-3	No noticeable effect	
PENICILLIN	150	60	84	108	8	131
STREPTOMYCIN	21	8	12	15	6	15
TETRACYCLINES	110	49	70	81	2	98
CHLORAMPHENICOL	30	16	18	22	6	24
TYROTHRICIN	24	0	0	1	20	2
NEOMYCIN	31	1	8	12	6	14
BACITRACIN	28	2	8	11	7	14
CHLORHEXIDENE	48	4	12	19	10	30
CHLORHEXIDENE + NEOMYCIN	36	10	16	23	4	30

* All the carrier strains were sensitive '<u>in vitro</u>' to the antibacterial agent used.

LENGTH OF TIME NASAL SWABS CULTURALLY NEGATIVE

FOR <u>STAPH. AUREUS</u> AFTER LOCAL APPLICATION OF ANTIBACTERIAL.

TABLE 82.

Number of Carriers	Daily Number of Applications to nares	Number of carriers treated Yielding Staph. aureus at end of treatment with Chlorhexidene	Chlorhexidene + Neomycin	Penicillin
6	6	1	0	0
6	4	2	1	0
6	3	4	2	0
6	2	4	4	2
6	1	6	5	3

Effect of number of Daily Applications of Antibacterial Cream on Recovery of Staph. aureus from Carriers' noses

TABLE 83.

Staph. aureus in the nares than the major antibiotics, both during the period of application to the nares, and the period following treatment. Thus the great majority of these treated carriers were recolonised after 4 weeks.

An increased effect was obtained by using various mixtures of these antibacterial agents and antibiotics. The most potent mixtures examined were those of chlornexidine and neomycin and a furazone compound with bacitracin.

The success attending the use of this method to suppress the nasal staphylococcus depends a great deal on the care taken by individual carriers in applying the cream regularly to the nares. It is probable that fewer applications of the cream would be more popular and so experiments were carried out to assess whether the antibacterial effect was sufficiently strong and prolonged if fewer than 6 applications a day were made to the nares.

10: The effect of reducing the number of applications of antibacterial nasal cream on the suppression of Staph. aureus.

Groups, each of 6 persistent nasal carriers of Staph. aureus were treated for 10 days with nasal cream containing antibiotic or antibacterial substance, the number of daily applications varying from 1 yo 6. Sampling swabs were taken from the nares of these carriers during and after treatment to assess the suppression of staphylococci.

The results (Table 83) indicate that the suppressive effect of /

of the antibacterial creams used was impaired when the number of daily applications was less than three when a potent antibiotic such as penicillin was used, and less than six for the less potent antibacterial agents such as chlornexidene.

11: Scheme for the use of antibacterial nasal creams

From the results of the foregoing experiments the following method of using the cream to suppress Staph. aureus in nasal carriers was followed.

1: Persistent nasal carriers were identified by bacteriological examination which consisted of 3 nasal swabs at intervals of a week examined for Staph. aureus.

2: The antibacterial agent chosen for use was shown to be active against the carrier strain of staphylococcus by in vitro susceptibility tests. This was more important when the major antibiotics were contemplated for incorporation in the cream. It was unnecessary if substances like chlornexidene or furazones were to be used.

3: The appropriate antibacterial cream was dispensed in $\frac{1}{4}$ oz. pots, 4 grammes being sufficient for 2 weeks application.

4: The carriers were instructed to apply the cream to the nostrils at least 6 times per day for a period of 10 days.

5: Bacteriological examination of nasal swabs was carried out 4 days after stopping the cream.

12. The Dissemination of Staph. aureus from nasal carriers following Treatment with antibacterial nasal creams .

The previous experiment has shown that no Staph. aureus was recovered from the majority of the nose swabs used to sample the anterior nares of carriers who had been treated with antibacterial nasal cream. If no staphylococci could be recovered in this way it was unlikely that many would be disseminated from the nose to contaminate the rest of the surface of the carrier, his clothes, handkerchief or environment. To show that this was so, four carriers who had been examined for dissemination of Staph. aureus by the methods described on page 154 , were re-examined after treatment with antibacterial nasal cream.

The nasal carriers were tested for the number of Staph. aureus liberated after 5 minutes of minor body movements, 10 uses of the handkerchief and contamination of the hands. After starting treatment the carriers were asked to change their under garments and put on a suit which had been recently dry-cleaned, and to wear this suit continuously for the remainder of the period of observation. The results (Table 84) indicate that there was a marked reduction in the number of Staph. aureus disseminated from these carriers when the nose swabs were negative for Staph. aureus. The cleaned clothes did not become appreciably impregnated with Staph. aureus during the period of the experiment, which /

which lasted about 6 weeks for each carrier.

The handkerchief still yielded Staph. aureus in 2 of the 4 cases examined, though the numbers were small compared with those recovered before treatment. The same was true of the number recovered after washing the hands in broth, as the organism was still recovered from 3 of 4 carriers, but in only 1 per cent of the former numbers. No Staph. aureus were recovered from objects touched by the treated carriers' hands.

These experiments show therefore that there was a greater than 90 per cent reduction in the number of Staph. aureus disseminated from carriers as a result of treatment to suppress their nasal staphylococcus.

NATURE OF EXPERIMENT	NUMBER OF STAPH. AUREUS RECOVERED BEFORE TREATMENT FROM CARRIER					AFTER TREATMENT FROM CARRIER				
	1	2	3	4	Av.	1	2	3	4	Av.
5 minutes minor body movements	32	47	132	28	60	0	2	0	5	2
1 use of handkerchief	1636	747	214	68	666	60	23	0	0	21
Washing of hands in 100ml. broth.	250000	175000	6000	70000	125000	200	1000	12	0	303
Touching dry object.	0	*	2	18	7	0	0	0	0	0
Touching wet object.	23	79	8	26	34	0	0	0	0	0

* =Not tested.

DISSEMINATION OF STAPH. AUREUS FROM NASAL CARRIERS BEFORE AND AFTER TREATMENT WITH ANTIBACTERIAL CREAM.

TABLE 84.

13. The Control of Nasal Staphylococcus aureus in Hospital Staff and its Effect on the Rate of Cross-Infection.

This investigation took place in a small general hospital of 170 beds in which there had been sporadic staphylococcal cross-infection which had been investigated in detail for a period of a year. The details of the staphylococcal infections occurring in the patients and the nasal carriage among the hospital personnel have been fully discussed above (Page 197).

1: The Incidence of Infection

From January 1953 until January 1954 there were 229 patients with infections from which Staph. aureus was isolated ; of these 138 were contracted in hospital and 91 contracted outside hospital. The monthly incidence during this period was fairly constant but for a seasonal fall during the middle of the summer which was correlated with a relatively low nasal carriage rate among the staff. (Fig. 17).

2: Nasal Carriage rate among the hospital staff.

This was 31 per cent in the middle of the summer of 1953, rising steadily to 42 per cent at the beginning of February, 1954 (Fig. 18).

The relationship between these carriers and cases of infection was established by demonstrating the similarity of the strains of Staph. aureus isolated from the patients' lesions /

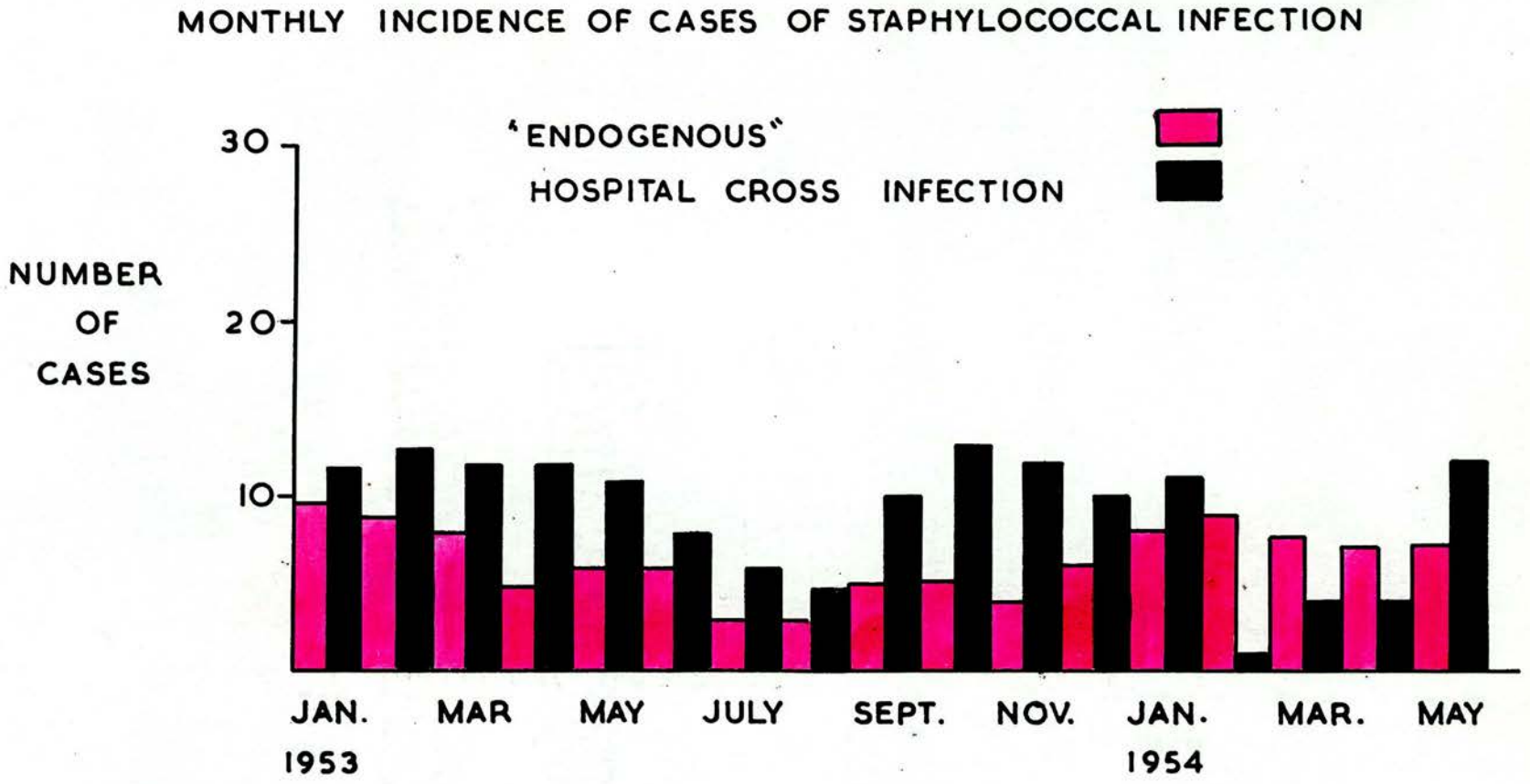
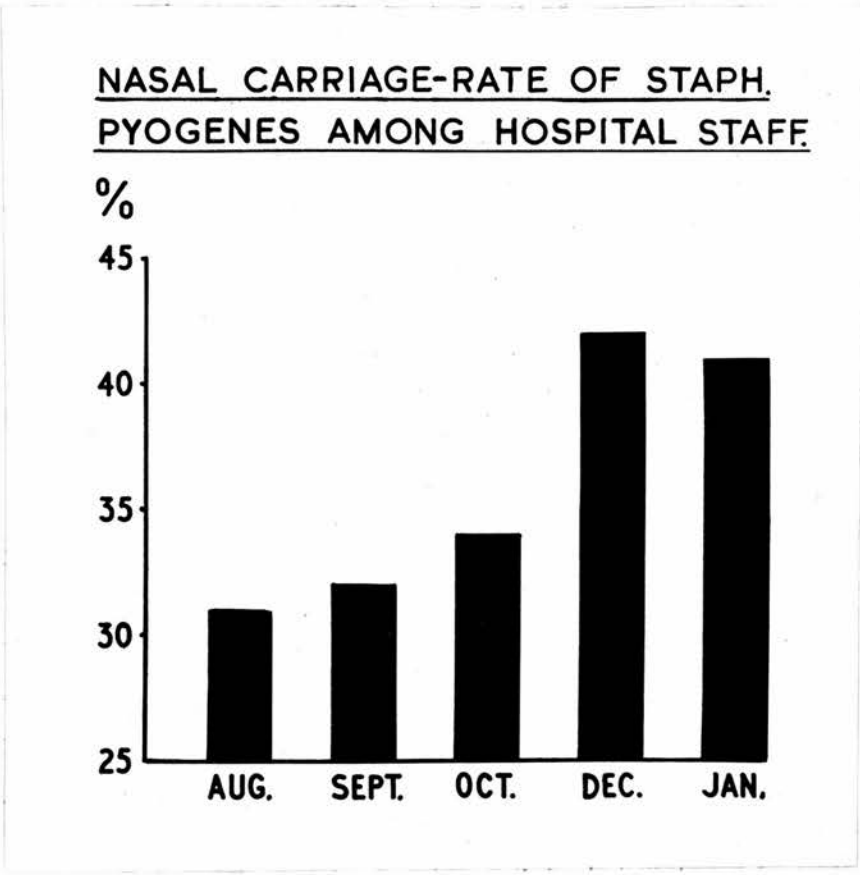

MONTHLY INCIDENCE OF CASES OF STAPHYLOCOCCAL INFECTION
"ENDOGENOUS"
HOSPITAL CROSS INFECTION
NUMBER OF CASES
30
20
10
JAN. 1953
MAR
MAY
JULY
SEPT.
NOV.
JAN. 1954
MAR.
MAY

FIGURE 17.



Nasal carrier rate among staff of Leith Hospital from August,1953 until January, 1954.

FIGURE 18.

lesions and the hospital staff carriers, in respect of susceptibility to bacteriophage and antibiotics. It was hoped to show the relationship further by controlling nasal staphylococci in the carriers and observing the effect of this on the incidence of hospital contracted staphylococcal lesions.

This experiment was commenced in February, 1954 by treating as many of the known nasal carriers in the hospital staff as possible with one course of antibacterial nasal cream. The suppression of Staph. aureus in the noses of these carriers and its rate of recolonisation was observed till the middle of the summer and correlated with the incidence of hospital contracted staphylococcal infections.

3: Antibiotic susceptibility of the Carrier strains of Staph. aureus

To ensure the selection of an effective antibacterial agent the minimum inhibitory concentration of the antibiotics was determined for each strain.

In this particular experiment, where a large group of individuals was involved, it was obviously of administrative advantage to select a single agent for use in all cases. This was facilitated in the present experiment as no strains of Staph. aureus resistant to the tetracyclines was observed among the carriers and so oxytetracycline was selected as the antibiotic of choice. Practically none of this antibiotic was being used in the hospital at this time and because of its known efficiency in suppressing nasal /

nasal staphylococci (Table 80) it seemed a good choice. The possibility of the drawbacks to the use of antibiotics was kept in mind and one aim of the experiment was to observe any change in the nature of the nasal flora following the use of this topical antibiotic.

4: Antibacterial cream for suppression of nasal staphylococci

A cream containing 1 per cen by weight of oxytetracycline was prepared and given to 34 nasal carriers. They were instructed to apply the cream 6 times per day to both nostrils for 2 weeks.

5: Results of treating Hospital Carriers

Subsequent cultures of nose swabs taken 2 weeks after withdrawal of the cream showed that only 3 of the 23 carriers were positive for Staph. aureus. Four weeks after withdrawal 9 carriers had positive cultures; 10 weeks after 13 were positive, 15 weeks later 19 carriers were positive, and in 20 weeks 26 carriers. Therefore, eventually nearly all the carriers became recolonised. Thus the overall carriage rate in the hospital was materially reduced for about 3 months, though this effect was not so obvious because of the arrival in the hospital of new nurses who had not been treated with the antibacterial cream. Because of this the nasal carriage rate at the end of May was 31 per cent (Fig.19).

The majority of the treated carriers recolonised with a strain of Staph. aureus identical in bacteriophage and antibiotic susceptibility to the original. In 6 of the 26 known to have recolonised /

EFFECT OF ANTIBIOTIC CREAM ON CARRIAGE OF STAPH. PYOGENES

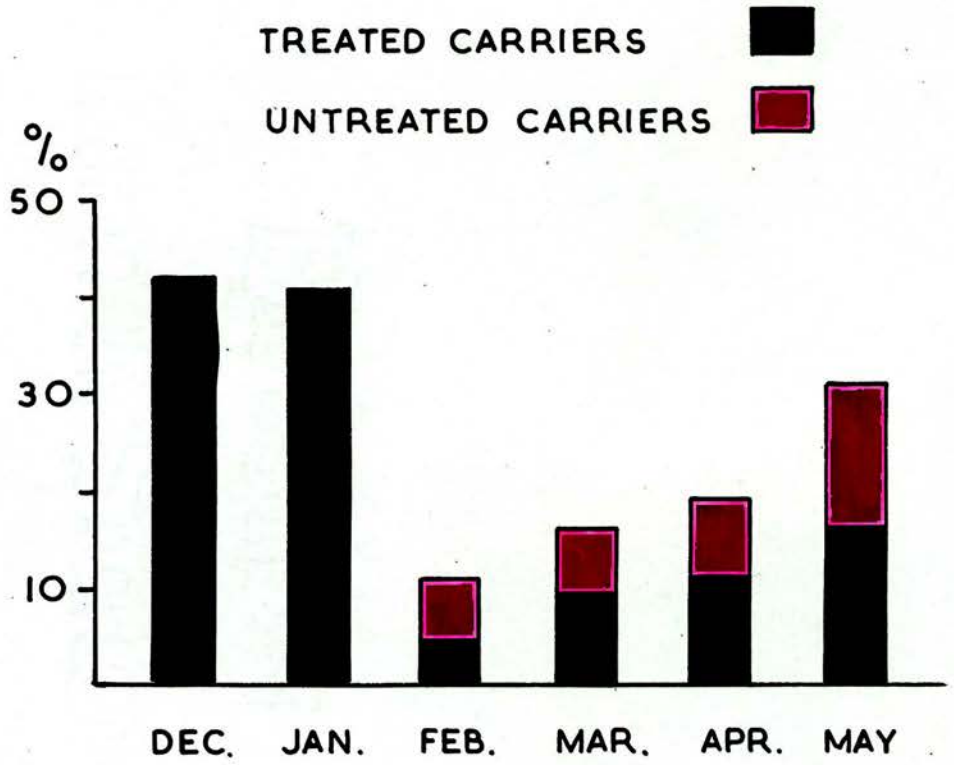


FIGURE 19.

recolonised however the strain of Staph. aureus was different in phage type and antibiotic susceptibility. In three, carrier strains originally of Group II pattern were replaced by strains with patterns of Group III of the "hospital type". In the other three the original "hospital type" strain of Group III pattern was replaced by a strain of Group I pattern. These replacement strains were all resistant to penicillin.

During the period of observation following the course of treatment with the antibacterial nasal cream new additions to the staff introduced a number of carriers who were not treated with the cream. In this way 12 strains were introduced by the end of April, so that the number of nasal carriers was 31.

Thus the use of the suppressive cream materially reduced the number of nasal carriers during February, March and April, but by the month of May the overall carrier rate was back to the pre-treatment level and the proportion of hospital-type strains was re-established, apparently by colonisation of the newly arrived personnel with the hospital strains.

There were no examples of oxytetracycline resistance among strains of Staph. aureus in this experiment. In none of the carriers was there a gross or permanent change in the quality of the nasal flora, apart from that noted for Staph. aureus. No untoward pharmacological effects of the cream were reported by the treated carriers.

6: Effect on the Number of Cases of Hospital Cross-Infection.

The reduction in the hospital staff nasal carriage rate was paralleled by a drop in the number of cases of hospital infections. (Fig.20). During the period preceding the administration of antibiotic there was a monthly average of 12 cases. In February, the first month of the experiment, there was only 1 case; in March and April there were 4 cases each month, in May 8 and in June 11. These figures strongly suggests that the fall in the rate of infection was due to the reduction of the carriage rate subsequent to the use of antibiotic cream, and this is substantiated by the following facts.

1: The nasal carriage rate among staff was high before the experiment and had risen with the number of cases of hospital infection during the autumn and early winter.

2: As the carriage rate increased after the experiment so did the number of infections.

3: The hospital infection rate was high, and more or less constant before the experiment, and from a comparison with the previous year would be expected to continue at a high rate.

4: The number of cases of non-hospital infection remained unaffected by the experiment, so ruling out a seasonal influence as occurred in the summer of 1953.

No see table

The conclusions to be drawn from this experiment were that the application of the oxytetracycline nasal cream to carriers /

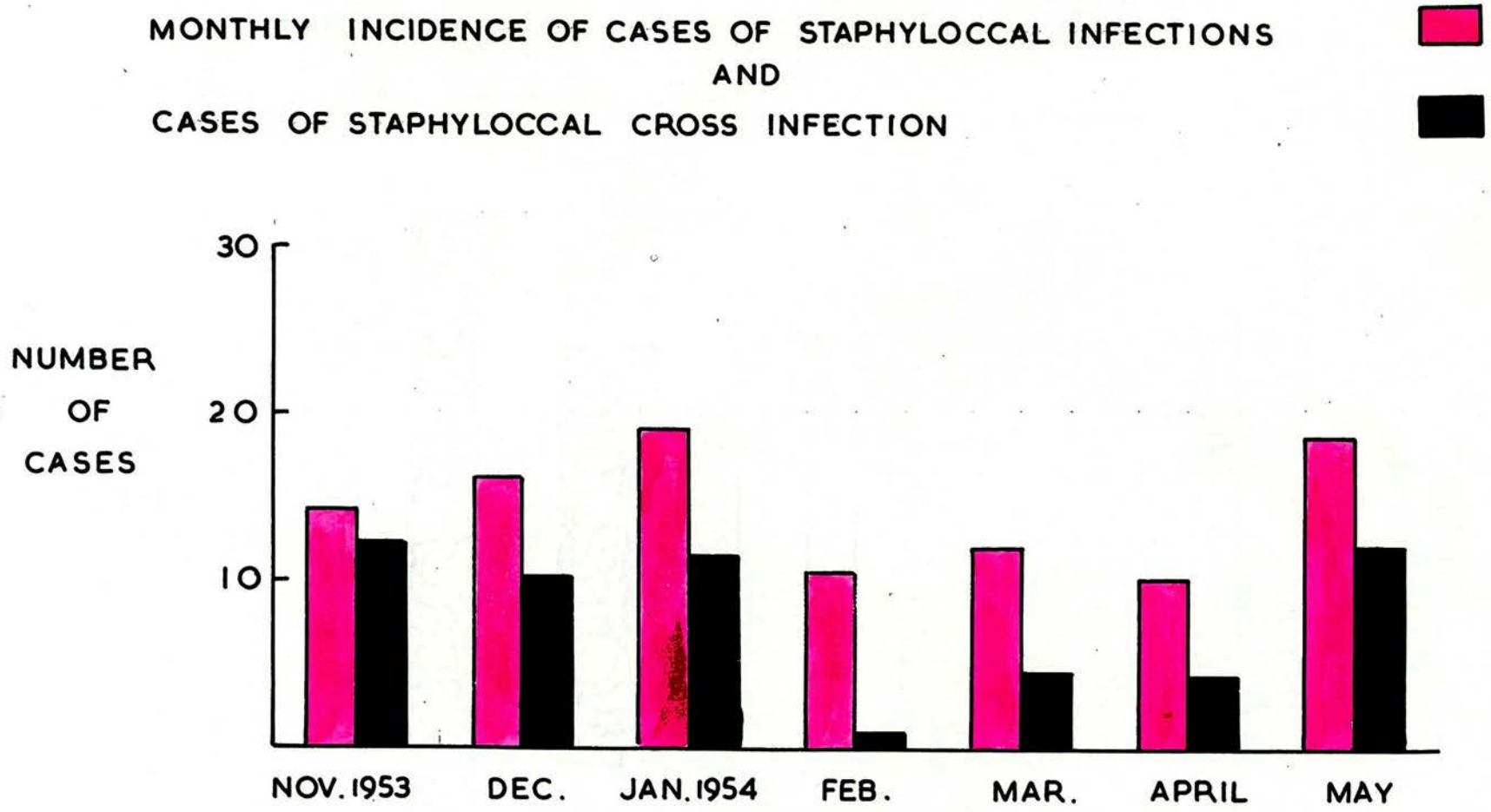

MONTHLY INCIDENCE OF CASES OF STAPHYLOCCAL INFECTIONS
AND
CASES OF STAPHYLOCCAL CROSS INFECTION
NUMBER OF CASES
30
20
10
NOV. 1953
DEC.
JAN. 1954
FEB.
MAR.
APRIL
MAY

FIGURE 20.

carriers of oxytetracycline-susceptible strains of Staph. aureus reduced the nasal carrier rate, and therefore the infectivity of more than 80 per cent of the treated carriers for 1 month, and of 50 per cent for 3 months. This resulted in an appreciable fall in the number of cases of hospital infection due to the staphylococcus.

14. The Effect of Treatment of Nasal Carriers among Maternity Unit Personnel on the Colonisation of Babies with Staph. aureus.

1: The high rate of colonisation of new-born babies in a maternity unit has already been noted (Page233). Since the strains colonising the babies were similar in their biological characters with those isolated from some of the staff carriers it was decided to test the effect of suppressing Staph. aureus in the carriers, on the rate of colonisation of the babies.

The colonisation rate of the babies on discharge was more than 87 during January and February, 1958 ; 81 in March and 64 in April (Table 74). The staff nasal carrier rate was 56 per cent in December and January, 60 per cent in February and 61 per cent in March (Table 74).

2: Antibacterial susceptibility of Carrier strains of Staph. aureus.

All of the strains isolated from the carriers were resistant to penicillin and 70 per cent were also resistant to streptomycin. Large percentages were also resistant to chloramphenicol and the tetracyclines. Although none of the strains was resistant to erythromycin it was considered very bad policy to introduce this antibiotic for an experiment of this nature. For this reason it was considered desirable to use an agent not likely to be generally exhibited in the maternity unit.

The antibacterial preparation used in this experiment was a mixture of furazone and chlornexidene and was given to all 18 known /

known carriers in the ward. All the strains of Staph. aureus isolated from staff carriers and babies in the ward were susceptible to the antibacterial effect of this preparation as shown by in vitro tests. The carriers were instructed to apply this preparation at least four times a day for 10 days. Four days after application of the nasal cream had stopped all the staff were again swabbed and again 2 weeks later.

3: Results of Suppressing nasal Staph. aureus in maternity ward staff.

Four days after stopping treatment with the antibacterial cream all of the carriers gave nose swab cultures negative for Staph. aureus. Two weeks later 3 carriers had recolonised. A month later there were 9 carriers partly due to a change in staff.

Prior to the experiment on the nasal carriers among the staff, 65 per cent of the babies were colonised in the anterior nares and naso-pharynx with Staph. aureus. During the month when the staff carriers were treated with the antibacterial cream the colonisation rate of the babies fell to 28 per cent but returned to 60 per cent in June. This was suggestive evidence that the temporary control of nasal carriage in the attendant personnel reduced contamination and colonisation of the new-born. This was supported to a certain extent by the observation that the number of Staph. aureus contaminated particles in air-borne dust in the maternity unit fell from an average of 12 per 100 cubic feet to 9 per 100 cubic feet after the experiment.

15. Effect of Treating nasal Staphylococci on the Recurrence of Staphylococcal Infection.

The incidence and nature of staphylococcal infection in general practice has already been referred to. More than half of the patients reporting with lesions had a history of recent recurrences and the very frequent association of nasal carriage in these persons and similarity of their nasal and lesion strains makes it likely that recurrence of lesions could be prevented by vigorous treatment of the nasal staphylococcus with antibacterial creams.

From January 1955 to December 1958, 376 cases of infection were examined for the first time (Page 211). Of these 284 became available for regular follow-up and 187 received antibacterial nasal creams to suppress their nasal staphylococci.

The antibacterial agent was chosen for each individual patient on the basis of the results of the *in vitro* susceptibility tests on the carrier strains of *Staph. aureus*. All the available antibacterial substances were used as indicated and no general restriction on the use of antibiotics was imposed since the conditions in general practice under which they would be used were somewhat different from those pertaining in hospital. The spread of antibiotic resistant strains from patient to patient was unlikely, and contamination of the environment with the antibiotic relatively slight.

During /

During the first year of this experiment all patients with a penicillin-susceptible carrier strain were given 1 per cent penicillin cream and those with a penicillin-resistant strain an alternative preparation. During subsequent years larger numbers have been given chlornexidene and furazone preparations, with and without admixture of neomycin and bacitracin, since no strains resistant to any of these agents were isolated from any of the patients.

2: Results of suppression of nasal staphylococci in cases of staphylococcal infection.

The result of treatment in 187 cases was measured by comparing the frequency of lesions before and after treatment with the anti-bacterial nasal creams. There were considerably fewer lesions occurring in these individuals in a two year period following treatment than in an equal period before treatment in each individual. Thus during the 2 year pretreatment period there was a history of 454 episodes of staphylococcal infection in 187 cases, while in the post-treatment period of 2 years, in most cases, there were only 87 episodes among the same cases.

Considered individually 153 cases had no further lesions ; the remainder had some recurrences but there were less frequent than before treatment, or subsequently improved as a result of further courses of treatment in 15 cases, and 19 cases can be said to have had little or no benefit.

3: /

3: Action of antibacterial cream on the Staph. aureus in the anterior nares.

In all those treated with the antibacterial cream there was a marked fall in the number of colonies of staphylococci isolated from nose swab cultures taken shortly after stopping treatment. Two weeks after stopping treatment no colonies of Staph. aureus were isolated from 142 of the 187 cases. Penicillin and the other major antibiotics were most effective in suppressing the staphylococcus so that in 70 per cent the organism was not recovered for 4 weeks, and in 30 per cent for 12 weeks. The results with chlornexidene combined with neomycin and bacitracin were almost as good, but those with chlornexidene or furazone alone definitely not so good.

In 21 of 187 cases receiving antibacterial cream strains of Staph. aureus of different antibiotic susceptibility than the original carrier strain were recovered and these were all changes in resistance to penicillin irrespective of the antibacterial agent used.

In 11 cases the original penicillin-sensitive strain was suppressed and replaced with a penicillin-resistant strain, usually of different phage 'type'. In 6 cases the change was temporary and within a few weeks the original penicillin-susceptible strain had fully re-established itself. However, in the remaining 8 cases the resistant strain persisted as long as the case was observed.

On the other hand in 7 cases, whose carrier strains were penicillin-resistant, suppression was followed by recolonisation with /

recolonisation with a penicillin-sensitive staphylococcus (after treatment with chloramphenicol or oxytetracycline), but there was no concomitant change in susceptibility to the antibiotic used. The other biological details of these strains are given in Table 85.

The results of these experiments to control Staph. aureus in the anterior nares of carriers substantiate the findings that there is a close association between the carriage of Staph. aureus and infections . The carrier may spread the organism to infect himself, or directly or indirectly to other persons. The suppression of Staph. aureus in the carriers at the site of growth is therefore a means of preventing infection for few, if any, organisms are available to contaminate the carrier or reservoirs in the environment.

The treatment of carriers, whether nasal or at some other primary site, should be sufficient in the home environment to markedly reduce the number of Staph. aureus in the environment; recontamination from other sources will be slight and the removal of the staphylococcus through death of the survivors and physical cleansing should control their numbers.

In hospital, the carrier is only one of the major sources, the other being the patient or member of staff with lesions. Reduction of Staph. aureus in the environment can only be achieved when removal and death of the organism exceeds replishment from the sources. At present in most hospitals, replenishment of reservoirs is more often in excess of the rate of removal so that there is an accumulation of Staph. aureus in the environment.

Under /

Under circumstances where there are few lesions, the treatment of carriers to suppress Staph. aureus may be sufficient to reduce the replenishment of the environment below the rate of removal and so the circle of reinfection and recontamination of new hosts may be broken. In other instances where there are cases of staphylococcal infection present, it will be necessary to isolate them and take measures to prevent the dissemination of the staphylococci into the environment. These may include barrier nursing, strict aseptic technique of carrying out dressings, effective specific therapy and specific measures to reduce the number of organisms in the environment.

Original Strain		Antibacterial Agent Used	Replacement Strain		Strain Finally Observed	
Phage Type	Penicillin Sensitivity		Phage Type	Penicillin Sensitivity	Phage Type	Penicillin Sensitivity
NT	PR	C	42E	PS	42E	PS
52/52A	PS	P	7/47/54	PR	52/52A	PS
3A/3B/3C	PS	P	3A/3B/3C	PR	3A/3B/3C	PS
53/76	PR	T	52	PS	52	PS
3A/3B/3C	PS	P	3A/3B/51	PR	3A/3B/51	PR
42E/47	PS	P	42E/47	PR	42C/47	PS
52A	PS	P	52A	PR	52A	PS
3A/3B/3C	PS	P	3A/3B/3C	PR	3A/3B/3C	PR
3A/3C	PS	P	42B/52	PR	42B/52	PR
NT	PS	Hib	7/47/75/77	PR	3A/3B	PS
3C/51	PS	Hib	77	PR	77	PR
54	PS	P	47/47A/54	PR	47/47A/54	PR
52/52A/79	PR	C	47/47C	PS	47/47C	PS
3A/3B/55	PS	Hib	NT	PR	3A/3B/55	PS
79	PS	P	29/52/52A	PR	29/52/52A	PR
53/54/77	PR	O	3A/3B/3C	PS	3A/3B/3C	PS
29/52	PS	P		PR		PR
77	PR	O		PS		PS
3B/51	PS	Hib	3B/51	PR	3B/51	PR
47/47C	PR	C	3A/3C/55	PS	3A/3C/55	PS
52A/80	PR	C	NT	PS	NT	PS

Changes in carrier strains immediately or shortly following treatment with antibacterial creams

TABLE 85.

THE ORIGIN OF ANTIBIOTIC-RESISTANT STAPH. AUREUS

It is generally believed that antibiotic-resistant variants of bacteria arise by mutation which may be spontaneous or induced. Following this initial step the maintenance and spread of the resistant variants in the environment will depend on favourably selective factors in the environment. Thus an antibiotic-resistant variant has a greater chance of survival than a sensitive one when growing in the presence of the antibiotic. If the antibiotic resistant variant possesses some other characteristics, different from those of the antibiotic sensitive form, these may also determine whether or not selection takes place in a suitable environment. Thus it has been supposed that antibiotic resistant variants have better growth properties or are more virulent in the tissues than the sensitive forms, but there is little satisfactory evidence that this is the case.

The appearance of resistant cells by mutation would explain the great increase in the proportion of antibiotic-resistant strains found in patients since the introduction of antibiotics for general clinical use. Alternatively patients may be infected with a mixture of cells, some of which are resistant and these will be selected out by the antibiotic; or the patient may be reinfected by a resistant strain during treatment.

Several reports have indicated the probable appearance of penicillin resistant Staph. aureus during treatment in patients (Rammelkamp and Maxon 1942 ; North and Christie 1945; 1946 ; Plough 1945, Blair et al 1946) though many of these have lacked sufficient /

sufficient evidence of identity of the sensitive and resistant strains. Thus Barber (1947) reported the appearance of resistant Staph. aureus following treatment in patients, but the resistant strains were of different phage type from the sensitive strains originally isolated.

The majority of phage snsceptible strains of Staph. aureus have been classified into the three major groups depending on reactions with corresponding groups of phages. The strains of different phage groups have been considered unrelated, and a patient originally infected with an antibiotic-susceptible strain of one phage group, and from whom a resistant strain of different group is isolated, is thought to have suffered a second infection from another source.

If antibiotic-resistant strains originated by mutation in sensitive strains under impact of the antibiotic during treatment one would expect the distribution of resistant strains between the various phage groups to be the same as that of the sensitive strains from which they were derived, but generally this investigation has shown this is not the case and it has also been shown that the number of antibiotic-resistant strains in hospital is strictly limited.

The origin of the drug-resistant strains occurring in the community is believed to be due largely to spread by cross-infection rather than by mutation from drug-resistant strains in infected individuals. Also, since a few strains isolated before the introduction of penicillin have been shown to be penicillinase producers it has been thought that the frequent occurrence of such strains to-day is due mainly if not entirely, to elimination of the sensitive organisms by the use of antibiotics, followed by the spread of the resistant strains.

It has already described that nasal carriage of penicillin-resistant staphylococci is significantly greater among individuals who have been treated with the antibiotic than among those who have not. Thus it would appear that the antibiotic is important in the maintenance of resistant forms of the organism in treated persons.

Several reports have agreed that there is a correlation between the incidence of antibiotic-resistant strains in the environment and the amount of antibiotic used. (Lepper et al 1953, Hoffs, Wisseman and Whelan 1954)

However a peculiar and significant finding is that the proportion of penicillin-resistant staphylococci in hospitals is increasing, not only among these strains isolated from patients treated /

However a peculiar and significant finding is that the proportion of penicillin-resistant staphylococci in hospitals is increasing, not only among these strains isolated from patients treated with penicillin, but also among those found in the noses of healthy carriers among the medical and nursing personnel, most of whom are not under treatment with the antibiotic. Thus more than 90 per cent of the nasal carriers in a hospital may harbour penicillin-resistant Staph. aureus and new personnel entering hospital with carrier strains which are penicillin-sensitive, are for the most part, rapidly colonised with these penicillin-resistant strains.

It has been suggested that these penicillin-resistant strains are acquired from patients and carriers by contact and cross-infection, but it is unlikely that the penicillin-resistant strains are more suited to colonisation of the healthy nose than the penicillin-sensitive form, for if this were so, one would expect their spread among the general non-hospital population. The evidence over the last few years shows no evidence of an increase in the proportion of penicillin-resistant staphylococci among the general population, comparable to that in hospital.

A possible explanation is that the nurses and medical staff receive enough antibiotic on their hands and fingers, and from the air, to maintain a selective concentration in their noses which inhibits the penicillin-sensitive staphylococci. If this factor is important one would expect that any community exposed to the presence of antibiotic in the environment would have a higher incidence /

incidence of antibiotic-resistant strains of staphylococci.

Confirmation of this possibility was sought by investigating the incidence of resistant strains in healthy carriers in an antibiotic contaminated environment which lacked treated patients as a source of the resistant strains. Such an environment was a factory which handled and dispensed penicillin.

Examination of Factory handling penicillin for penicillin-resistant Staphylococcus aureus

1: Nasal carriage of Staph. aureus in the factory.

The staff of this factory were first examined for nasal carriage of Staph. aureus. They were divided into the following groups.

(a) those working in contact with penicillin

(b) those working on the factory floor, but not directly with penicillin.

(c) administrative personnel who were working in a separate building and did not come into continuous contact with the factory itself.

At the same time a group of individuals from a general practice in the same geographical locality were similarly examined. These persons were chosen as having had no contact whatsoever with the factory nor its personnel.

The nasal carrier rate for these groups, and the antibiotic susceptibility and bacteriophage types of the carrier strains of Staph. aureus are shown in Table 86.

2: Nature of the carrier strains of Staph. aureus

The carrier strains from the factory personnel resemble those commonly found in hospitals, both in their resistance to penicillin, which was always due to penicillinase production, and the limited number of types present. The majority had patterns of Group III and were /

TABLE 86

Group	Number of persons examined	Percent. carriers of Staph. aureus	Average number of Staphylococci on nose swabs	Percentage of carrier strains				Penicillin recovered from 100 cub. ft. samples of air	Presence of Penicillin on Fomites	Presence of Penicillin in nares or hands of Staff
				Penicillin resistant	Phage Groups I	II	III			
(a) Penicillin Filling Rooms and Packing Rooms	44	9	+	100	25	0	75	Filling Room 10-25 µg. Packing Room 2-30 µg.	Large Amounts	Present in those examined
(b) General Packing Room (no Penicillin handled)	32	27	+++	100	36	0	60	Small amounts of Penicillin variable amounts of other anti-bacterial agents	Not tested	Not tested
(c) Office	28	32	+++	100	30	0	70	Traces of Penicillin	Not tested	Not tested
(d) General population outside factory	36	30	+++	12	27	64	9	-	-	-

Staph. aureus strains in factory and control groups. Amount of penicillin recovered.

were quite distinct from those strains isolated from the carriers among the general population, which in turn were typical of strains found elsewhere in the non-hospital population.

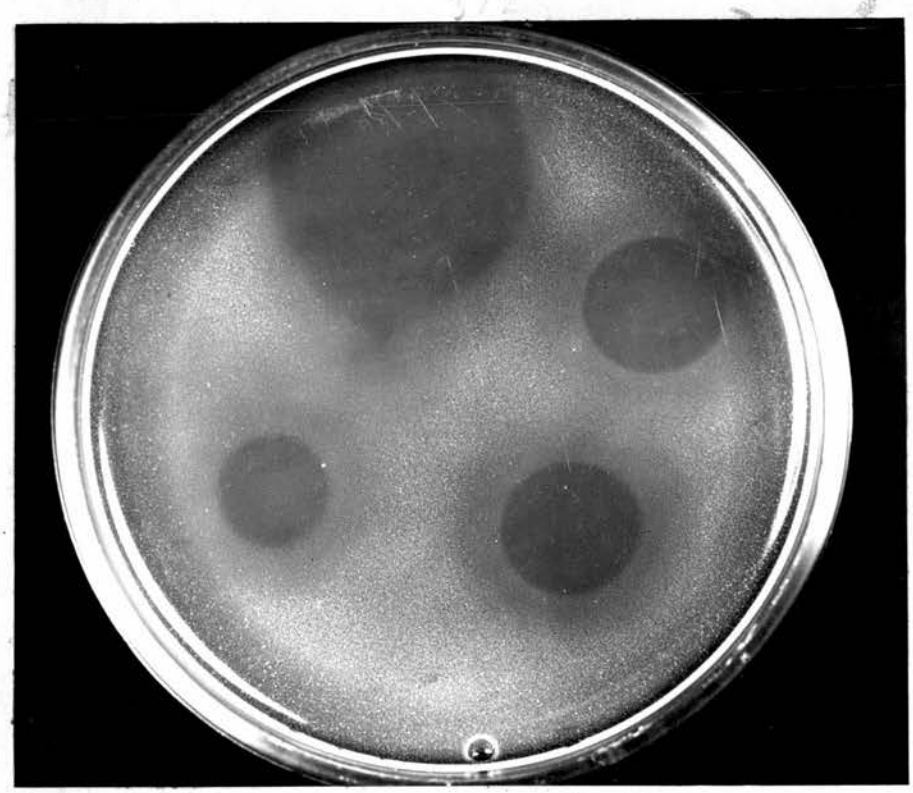
It was a striking fact that no penicillin-susceptible staphylococci, either coagulase-positive or negative, were isolated from any of the factory staff. It was also noteworthy that only small numbers of micro-organisms were isolated from the nares of persons working in close contact with penicillin, and this was correlated with a very low nasal carriage rate of Staph. aureus.

3: Recovery of Penicillin from the air in the factory

The next experiment was to measure the penicillin-content of the air at various sites in the factory.

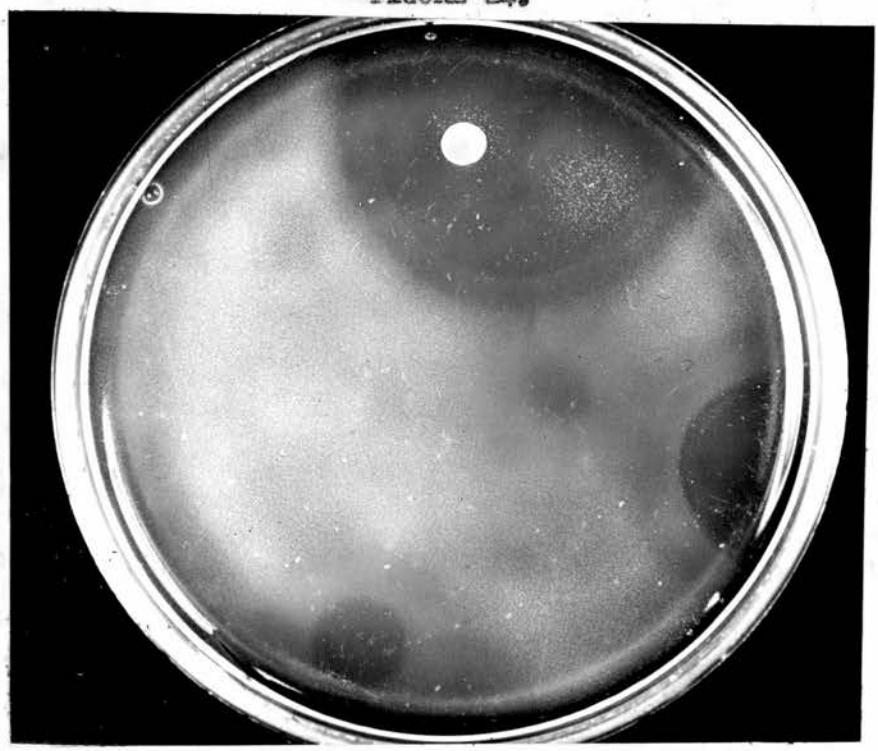
Penicillin was detected by means of 'indicator plates' (Fig.24) which were Petri-dishes cast with agar previously sown uniformly with a penicillin sensitive micro-organism (Staph. albus; Serratia marcescens and Strep. pyogenes) After incubation zones of inhibition were proved due to penicillin by inoculating a penicillin-susceptible staphylococcus on one part of each zone of inhibition, and this staphylococcus plus penicillinase onto an adjacent part (fig. 25). If inhibition was due to penicillin, growth of the staphylococcus on the penicillinase treated area only occurred. This proved that the inhibition was due to penicillin.

Using this technique the penicillin-content of the air in the factory /



Indicator plate (see text) after exposure to settling dust containing antibacterial activity The zones of inhibtion of growth are measured and the amount of activity calculated for the graph (Fig.26) if it is due to penicillin.

FIGURE 24.



Indicator plate exposed as in Fig.24 showing confirmation of penicillin as the inhibitory agent. A drop of penicillinase solution has been placed on the right hand portion of the large inhibition zone, and a drop of penicillinase and a drop of culture of a sensitive staphylococcus at the left hand side. Growth of the indicator organism can be seen.

FIGURE 25.

factory was measured by exposing indicator plates to settling dust particles ('settling plates'), and also by means of an impinger sampler taking in 25 cub. ft. of air per minute through 400 small holes onto the surface of an indicator plate (this was a modified De Buy and Crisp sieve plate sampler with a collection efficiency of 60 per cent for particles within the range 1 - 5 microns, and probably nearer 100 per cent for particles in the range 20 -100 micra.

The majority of the areas of inhibition of growth must result from a single particle of the antibiotic. The equivalent concentration of penicillin required to produce these zones of inhibition can be calculated, and thence on the basis that the constitution is pure procain penicillin, or the sodium salt of benzyl penicillin, an equivalent particle size can be calculated, as shown in the graph, (Fig. 26), which relates the zone of inhibition of growth to penicillin-content and size.

4: Experiment to measure the zones of inhibition on Indicator Plates

Indicator plates were prepared by casting suitable Petri-dishes with agar to a depth of 3.5 mm. Before pouring the agar was sown with two drops of an 18 hour culture of the penicillin-susceptible organism at 50°C., to ensure even distribution.

Pin-point drops of solutions containing known amounts of penicillin were placed on the surface of these plates and the zones of inhibition of growth measured after overnight incubation at 31°C. The graphs were constructed by plotting the diameters of the zones of inhibition against the penicillin content of the drops which were converted into equivalent particle sizes by calculation.

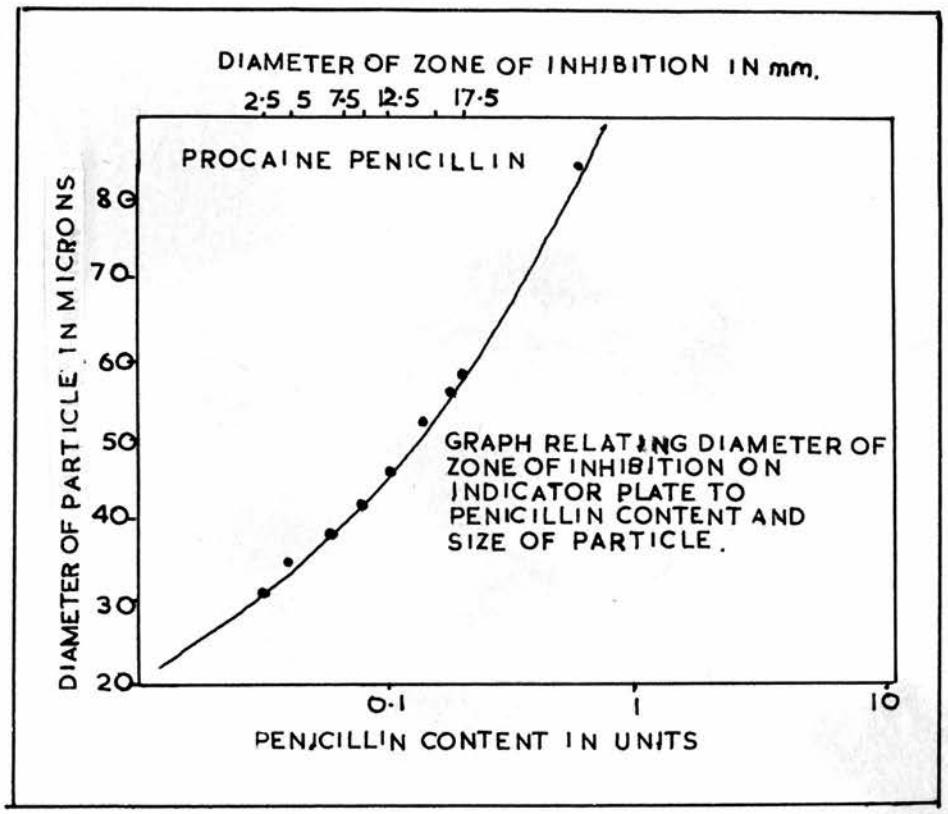

DIAMETER OF ZONE OF INHIBITION IN mm.
2·5 5 7·5 12·5 17·5
PROCAINE PENICILLIN
DIAMETER OF PARTICLE IN MICRONS
80
70
60
50
40
30
20
0·1
1
10
PENICILLIN CONTENT IN UNITS
GRAPH RELATING DIAMETER OF ZONE OF INHIBITION ON INDICATOR PLATE TO PENICILLIN CONTENT AND SIZE OF PARTICLE.

FIGURE 26.

The smallest amount of penicillin giving a definite zone of inhibition was 0.02 unit. This amount of procaine penicillin is contained in a sphere of approximately 36 micra in diameter, and of sodium benzyl penicillin, in a sphere 32 micra in diameter. Indefinite zones of inhibition were due to smaller particles, and those less than 25 microns in diameter would not give individual zones of inhibition, and would only be detected when enough of them aggregated at one place in the medium to produce recognisable inhibition.

The results of sampling the air in various parts of the factory showed inhibitory activity against test strains of bacteria recovered from several parts of the main factory building where penicillin was dispersed or handled. Inhibitory activity was also present in the air from parts of the factory where the antibiotic was not regularly handled, and as some of this could be neutralised with penicillinase it must be presumed due to penicillin. Much smaller amounts of antibacterial activity were detected in air samples obtained from the separate administrative building (Table 86).

5: Recovery of antibiotic from Factory Personnel.

The presence of penicillin on the surfaces of persons working in the factory was shown in the following ways

(a) Fingers and hands were tested for contamination with antibiotic by impressing them on indicator plates.

(b) The anterior nares was sampled with saline moistened swabs, and these were rubbed on the surface of indicator plates, or, alternatively small filter paper discs were placed on the surface of the skin lining the nostrils, and after a minute, transferred /

transferred to the surface of indicator plates. However the most successful method was to smear the skin lining of the interior of the nostrils with an emulsion cream, and after a suitable interval remove some of the cream with sterile paper discs and transfer them for assay to the surface of indicator plates.

6: The presence of antibiotics on the surface of Fomites

Fomites were sampled by (a) direct swabbing with moistened swabs and transferring to indicator plates, and (b) by touching objects with hands free of antibiotic and then impressing the hands on the surface of indicator plates.

7: Results of the experiments to demonstrate antibiotic in the Factory.

Appreciable amounts of penicillin were recovered from the air in several parts of the main factory building where penicillin was dispensed or handled.

Inhibitory activity against test strains of bacteria was present in the air from parts of the factory where penicillin was not regularly handled, and some of this could be neutralised with penicillinase and therefore must be presumed due to penicillin. Small amounts of antibacterial activity could be detected in samples of air from the administrative building. (Table86).

The concentration of penicillin present in the air of the penicillin-filling room and packing room appeared to be sufficient to /

to depress the growth of all species in the cases of persons working in that environment, and the strains of staphylococci isolated were all penicillinase producers.

Much smaller amounts of the antibiotic were recovered from the air of other parts of the factory, and though not sufficient to depress the growth of the nasal flora were sufficient to select and maintain penicillin-resistant strains of Staph. aureus.

Traces, or small amounts of antibacterial substances including penicillin reach the administrative block, and some of this will be in the air and dust, and on the clothing and fingers of the staff; thus the antibiotic could be transferred to the nares of persons and be effective in maintaining penicillin-resistant bacteria.

The evidence of these experiments confirms that penicillin in the environment is an important factor in selecting and maintaining penicillin-resistant Staph. aureus. Thus it is reasonable to compare the occurrence and distribution of these strains in this factory and a typical general hospital, and suggest that antibiotic in the environment is an import/ant factor in hospital leading to widespread colonisation of personnel with Staph. aureus. Investigations were therefore carried out to demonstrate antibiotic in the hospital air.

Experiments to demonstrate antibacterial activity in the environment of a general hospital

1: Recovery of Antibiotic. Most of the observations were carried out in a large general hospital (Royal Infirmary) which included some 900 medical and nursing personnel. Antibiotic was recovered on indicator plates as described in the previous experiment for recovery of antibiotic from a factory environment. Examinations of air and dust, fomites and persons working in the hospital environment were made.

2: Results of Air Sampling in Hospital

The results of sampling air and dust in various parts of the hospital showed that antibacterial activity was present in all sites tested though in variable amounts, and much of this was due to penicillin. (Table 88). The smallest amounts of penicillin were found in the doctor's side rooms where antibiotic was presumably never handled. Large amounts were recovered from medical and surgical wards, as well as some corridors. The air of the dispensary contained a great deal of antibacterial activity which was greatest near the room where dispensing of antibiotics was carried out. A proportion of this activity was due to penicillin. The highest levels however, were found in parts of the out-patient department, and this site was examined in greater detail. This site was especially suitable as penicillin was the only antibiotic in use at the time.

3: /

Hospital Site	Amount of Penicillin Settling on 12 sq. ins. per hour µg.	Penicillin Content of Air per 100 cub.ft. Measured by Sieve Sampler µg.	Presence of Penicillin on Fomites	Presence of Penicillin on Hands and in nares of Staff
Doctors' Rooms	0 - 0.02	Not tested	Not tested	Not tested
Corridor	0 - 0.05	Not tested	Not tested	Not tested
Medical Ward	0.06 - 0.1	trace-0.4	Not tested	Not tested
Surgical Ward	0.05 - 0.25	trace-0.5	Not tested	Not tested
Dispensary	1.0 - 1.7	0.5 - 35	Not tested	Not tested
Out-Patient Department				
Minor Surgery Rooms	1 - 5	1 - 50	Present	Present
Dressing Rooms	0.5 - 1.0	1 - 15	Present	Present
Corridor	0 - 0.15	trace-0.6	Not tested	-
Waiting Room	0 - 0.2	trace-1.0	Not tested	-
Small Ward	0 - 0.05	traces	Present	Present
Doctors' Room	0 - 0.05		Not tested	-
Operating Theatre	0	0	Not tested	Not detected

Environmental penicillin in hospital

TABLE 88.

3: Results of Examinations for Antibiotics in the Out-Patient Department

Within the department the amount of penicillin recovered varied greatly but was highest where penicillin was being administered, in the rooms used for minor surgery. During the period of this investigation very little penicillin was being used or given to patients in any other part of the department. The amount of penicillin recovered fell off rapidly as the distance from the incision room increased, and very small amounts were recovered from the doctors retiring room, or the operating theatre, which was georgraphically farthest removed. The values at all sites varied widely from time to time (Table 89).

4: Detection of penicillin on persons and fomites in the Out-patient department.

Fomites were handled, and the hands previously shown to be free of antibiotic activity were impressed on indicator plates. Amounts of penicillin sufficient to inhibit growth over the full plate were obtained in many instances after a few seconds handling of articles such as tray covers, dressing containers and bottles present in the dressing rooms and rooms used for minor surgery (Table 89).

Hands of the medical and nursing staff were examined before washing and at various times during the day. Amounts of penicillin sufficient /

sufficient to inhibit large areas of indicator plates were present on the hands and fingers of half of the doctors and nurses. Apparently these persons had picked up the penicillin from fomites by handling in the natural course of their duties. No attempt was made to estimate quantitatively the amounts present on the hands or fomites.

5: Results of examination of the anterior nares of nurses for penicillin

The anterior nares of some of the nursing staff were tested. This was a cruicial matter, for if environmental penicillin affects the growth of Staph. aureus it is most likely to do so in the nostrils, the primary site of colonisation in the carrier, and a selective concentration must be present, at least for certain periods.

The results showed that all members of the staff working in the incision and dressing rooms, and four out of eight working in other parts of the department and easily detectable penicillin in their nares after a morning's work. This penicillin could have been inhaled from the air, or introduced by contaminated fingers (Table 89).

6: Nasal Carrier Rates of Staph. aureus among the Out-patient Staff.

The persistent nasal carriers comprised 53 per cent of the staff of the whole hospital at this time, and 90 per cent of these carriers harboured penicillin-resistant strains (Page 133).

The nasal carrier rate of the staff of the out-patient department was in excess of 60 per cent, and all of the persons who were carriers harboured penicillin-resistant strains (Table 89). The number of colonies of Staph. aureus isolated from carriers in whom detectable penicillin was recovered from the nose was significantly less than in these carriers who had no detectable penicillin.

Individuals	Presence of Staph. aureus in nares	Penicillin susceptibility of nasal strain	Bacteriophage Types	Antibiotic presence in nose and/or hands
Surgeon 1	+++	Resistant	6/7/47/54	-
Surgeon 2	-			-
Surgeon 3	++	Resistant	52/52A/80	-
Surgeon 4	-			-
Surgeon 5	+++	Resistant	52/52A/79/80	Present
Surgeon 6	-			Present
Nurse 1	+++	Resistant	7/54/73/77	-
Nurse 2	++	Resistant	7/54/73/77	Present
Nurse 3	-			Present
Nurse 4	+++	Resistant	52/52A/80	Present
Nurse 5	+++	Resistant	52/52A/79	Present
Nurse 6	+++	Resistant	NT	-
Nurse 7	-		-	-

Nasal carriage of Staph. aureus among members of staff of hospital out-patients department and details of the carrier strains

TABLE 89.

Hospital Site	Amount of penicillin settling on 12 ins./hr. µg.	Penicillin per 100 cub. ft. of Air measured by sieve sampler µg.	Presence of Penicillin on hands of Staff
Duty Room	0 - 0.05	0 - 0.1	4 out of 8 persons had Penicillin on hands
Corridors	Not tested	0.1	
Wards	0 - 0.1	0 - 0.1	

Environmental penicillin in a maternity unit (Hospital W.).

TABLE 90.

7: Examination of a Maternity Unit for Antibiotic

This Unit was situated in another general hospital. (Western General Hospital). Sampling of the air and dust showed variable quantities of antibiotic activity up to 0.1 μg of penicillin per cub. ft. (Table 90)

Antibiotic activity was detected on the hands of 50 per cent of the nurses examined and on several occasions was in sufficient quantity to inhibit the growth of an entire indicatir plate.

8: Experimental Contamination with Penicillin

Experiments were carried out in laboratory rooms to show that contamination of the air and dust can occur when penicillin preparations are dispensed and handled as they are in hospital.

A hard surface was contaminated by spilling, dropping, squirting penicillin solution (sodium benzyl penicillin) and suspensions (procaine penicillin) in concentrations calculated to simulate those that would be used during the preparation and giving of penicillin injections, and the washing out of penicillin-contaminated syringes.

The preparation of an injection of penicillin, or other antibiotic usually involves adding a few millilitres of water to a phial of dry penicillin and a solution or suspension containing 300,000 to 600,000 units of penicillin per ml. is sucked up into a syringe. Our observations indicate that approximately 0.2 ml. remains in the syringe and needle after the injection, equivalent to 60,000-120,000 units, and this amount is diluted to a varying degree during cleaning out of the syringe.

Measurements /

Measurements were made before and after the contamination procedures by exposing indicator plates and sieve sampling. After the contaminated surfaces had dried, dust was raised by agitation and measurements continued. The results (Table 92) showed that contamination of a previously penicillin-free room occurred, and exposed indicator plates and sampler plates showed zones of inhibition of growth comparable to those obtained in the hospital environment. The frequency of occurrence of particles of different calculated sizes corresponded with those found in hospital and it can be seen that a greater proportion of large particles were detected on the settling plates than on the sampler plates. (fig.27).

Direct observation of the particles of penicillin in suspensions and crystals formed during evaporation of solutions showed that the sixe distribution corresponded to the distribution observed in hospital and in the contaminated laboratory room for particles of 30μ and more. There were many particles smaller than 30μ but these are too small to be detected individually on the indicator plates. This would seem to confirm that it was the particles and crystals from the penicillin preparations which contributed most to the contamination of the hospital environment. (fig.27).

If the air in the contaminated room remained still, and there was no further activity, the amount of penicillin recovered on settling plates and by sieve-sampling rapidly became less with time until after 25 minutes it was exceptional to recover any antibiotic. Disturbance of the air and dust produced recirculation of penicillin-contaminated particles and these could be recovered for a further 20-25 minutes, dependent on the degree and continuance of agitation. This was to be expected from the relatively large

size /

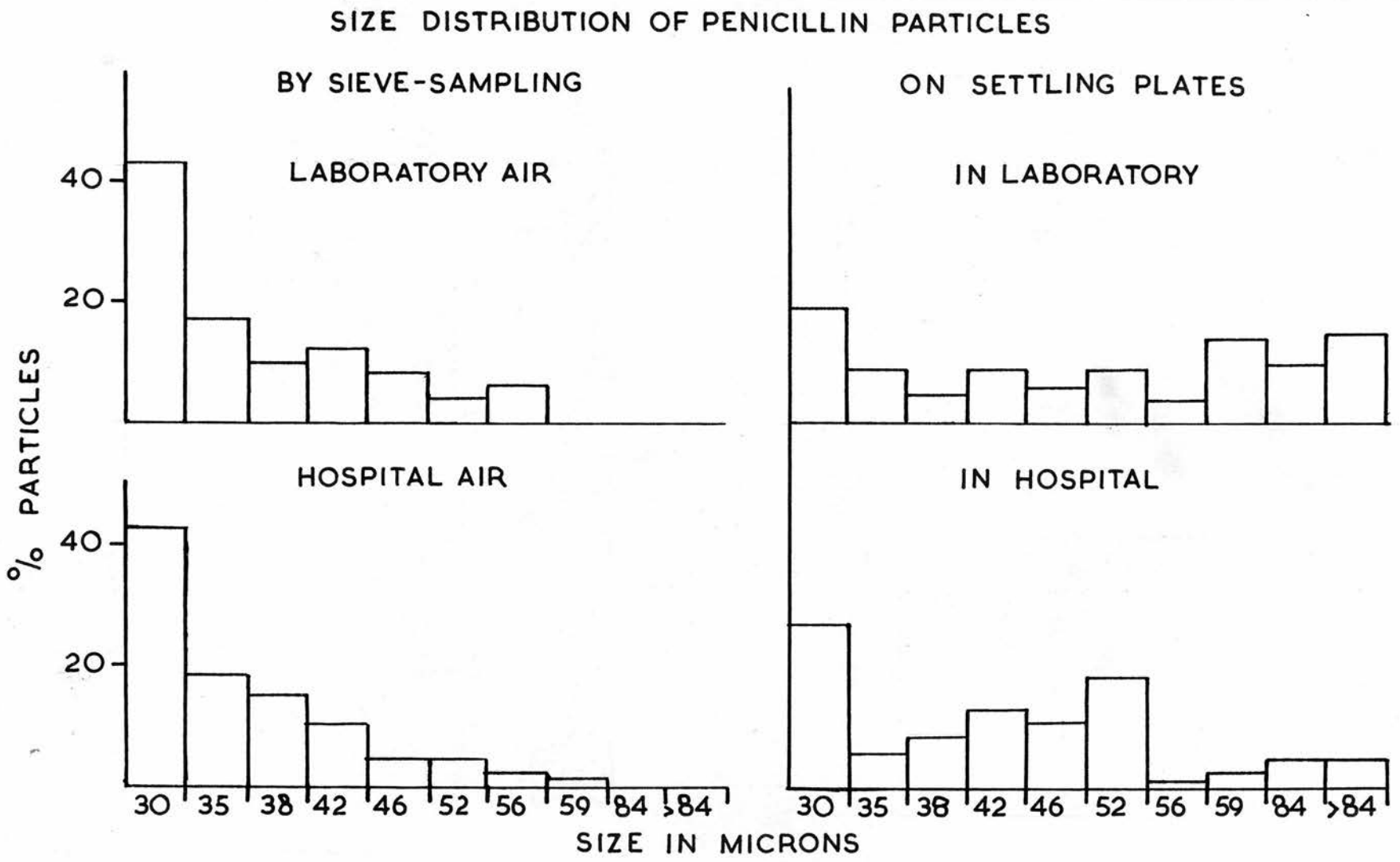

SIZE DISTRIBUTION OF PENICILLIN PARTICLES
BY SIEVE-SAMPLING
ON SETTLING PLATES
LABORATORY AIR
IN LABORATORY
HOSPITAL AIR
IN HOSPITAL
% PARTICLES
40
20
40
20
30 35 38 42 46 52 56 59 84 >84
30 35 38 42 46 52 56 59 84 >84
SIZE IN MICRONS

FIGURE 27.

	Time in Minutes Following Contamination or Reagitation	Penicillin settling on indicator plates of 12 sq. ins. area per hour at 5 metres distance from source of contamination.	Penicillin Content of 100 cub. ft. of air by Sieve Sampling
		µg	µg
Before Contamination	-	0	0
After Initial Contamination	1 - 5	2 - 5	10
	6 - 10	0.32	4
	11 - 15	0.11	2
	16 - 20	0	1.5
	21 - 25	0.02	0.05
	26 - 30	0	0
After Reagitation	1 - 5	5	5
	6 - 10	0.6	3.2
	11 - 15	0.1	1.1
	16 - 20	0.2	trace
	21 - 25	trace	0
	26 - 30	0	0
	30 - 40	trace	0

Recovery of penicillin in a room contaminated by 100,000 units of procaine penicillin

TABLE 91.

size of the penicillin particles involved, for those larger than 25 microns will settle to the ground and other surfaces in a matter of a few seconds (Duguid,1946).

9: Persistence of Environmental Penicillin

The room contaminated in the previous experiment was re-examined after 12 months to measure residual antibiotic activity in the dust.

Indicator plates were exposed to settling dust and the sieve sampler used to measure air-borne antibiotic. Small amounts of antibiotic activity, proved to be penicillin were detected by both methods after agitation of the dust in the room, but not before agitation (Table 92).

The board which had been contaminated and used as the source of the penicillin in the original experiment was still heavily contaminated with the antibiotic.

These facts demonstrate that penicillin in dry dust and protected from direct sunlight can retain its activity for at least one year. If there are regular contributions to the environment they may exceed the natural destruction of the antibiotic and thus there will be an accumulation of activity.

Time following agitation of the dust in minutes	Penicillin settling on indicator plates per 12 ins. per hour µg.	Penicillin content of 100 cub. ft. of air by sieve-sampler µg.
Before agitation	0	0
1 - 5	0.16	0.1
6 - 10	0.32	0.16
16 - 30	trace	0.05
30 - 60	0	0

Recovery of penicillin from room 12 months after contamination with penicillin

TABLE 92.

DISCUSSION.

This investigation has shown that staphylococcal infections are due to a large number of strains of Staphylococcus aureus which are derived almost exclusively from living sources. These sources are situations in which the organism is actually growing and multiplying and must be distinguished from the almost numberless sites in the environment from which Staph. aureus can be recovered and which are more correctly described as reservoirs. The reservoirs are contaminated with staphylococci from the sources through various routes. The reservoirs lose staphylococci by spread and dispersal to other parts of the environment, to new hosts, and by death and destruction during cleansing procedures. The number of staphylococci in a reservoir at any one time will depend upon the balance between rate of replenishment from sources, and the rate of removal.

It seems to be established that human beings are the chief source of the strains of Staph. aureus responsible for staphylococcal infections in man, and animals probably play a minor role in this respect. Staph. aureus may grow under natural conditions outside of the body, such as in foodstuffs that have been contaminated with the organism, and these will also form a source of the organism and its toxins in such conditions as staphylococcal food-poisoning. In general however, the number of Staph. aureus recovered from sites outside of the body suggest that significant multiplication does not occur.

In/

In human beings Staph. aureus grows profusely in a number of surface sites. Numerous investigators have found the anterior nares to be an important site of growth and this, the present investigation has confirmed. The accumulated evidence suggests that the nose is a site which is most important as a source of the organism for contamination of reservoirs in the environment and other hosts. It is also probably important in the ecology of the species sihce the anterior nares is exposed to many environmental influences which have an effect on the growth of staphylococci.

It has been shown that Staph. aureus can be recovered from the anterior nares of many persons but not all can be regarded as true carriers of the organism, that is, persons in whom the organism is growing and multiplying.

According to the observed frequency and duration of recovery of Staph. aureus carriers have been classified as persistent, intermittent or temporary. Persistent and inter-:mittent carriers are regarded as true hosts of this organism; in these individuals the staphylococci live and multiply in the nasal vestibule. By means of phage typing, the strains isolated on successive occasions from these carriers have been shown to be identical. The persistent carriers are easily recognised since the predominant organism in their nasal swab cultures is a pigmented, coagulase positive staphylococcus which is susceptible to lysis with one or more of the staphylo-:coccal phages, and there is very little variation in the relative/

relative numbers isolated on each occasion of sampling. There must therefore be a profuse growth of the organism in the nares of these carriers. Intermittent carriers do not have large numbers of staphylococci continuously present in their nares so that sometimes a swab proves negative for Staph. aureus on culture. At other times the number of staphylococci may be large. Some factor presumably prevents sufficiently large numbers of the organism coming to the surface for isolation at every swabbing.

A third group of persons also yielded Staph. aureus intermittently from their anterior nares and have been called temporarily contaminated persons, or temporary carriers. They are differentiated from true intermittent carriers by the fact that staphylococci isolated from them on different occasions are found to be of differing phage type. It seems that they filter off Staph. aureus from the inspired air so that it is picked up by the sampling swab and isolated on culture, almost always in small numbers.

It may be difficult to distinguish a temporary carrier from a persistent or intermittent carrier on the basis of a single examination, though a heavy growth of Staph. aureus in a nose swab culture is good presumptive evidence of a true-carrier state. Thus an estimation of the carrier rate in a group of persons, based on the results of a single observation may be inaccurate and considerably higher than that based on the results/

results of repeated examinations over a reasonably long period.

On the basis of such examinations the proportion of true nasal carriers of Staph. aureus has been estimated in various groups of the population. Among the general population, not in hospital, the rate is approximately 30 %, varying to a certain extent in different social and age groups. In contrast, the carrier rate in hospital personnel is usually significantly higher at 40 - 50 %, though not so high as has been frequently reported on the basis of a single swabbing.

The greater number of adults therefore do not harbour Staph. aureus in their anterior nares but may be temporarily contaminated with the organism at some time. Thus, of one group studied, 85 - 90 % had yielded the staphylococcus at least once over a period of 12 months. It has been supposed that these non-carriers differ in some fundamental respect from those who are carriers, and that they remain persistently free of Staph. aureus (Hutchison, Green and Grimson 1957). The results of the present investigation indicate that those persons who are carriers certainly remain so for considerable periods of time. Among the students examined this period averaged 14 years, assuming that the number of carriers who ceased to harbour the organism is more or less constant at different ages. In this same group, the number of persons previously non-carriers who became carriers of Staph. aureus equalled the number who ceased to be carriers over the same period, so explaining the relatively constant carriage rate observed

during/

during adult life. The balance between persons acquiring Staph. aureus and those losing the organism may not be the same in other age groups.

The new-born baby with sterile surfaces is contaminated within a matter of minutes by many and diverse species of bacteria including Staph. aureus. Within a few hours after birth Staph. aureus can be recovered from the nares, umbilicus, mouth, skin and faeces of some babies and from the majority within a week (Duncan and Walker, 1942). Within two weeks more than 90 % of hospital born infants may be colonised (Miles, 1941) though the comparable figure is lower in home births (Ludlam 1953; Hurst, 1957). These results have been fully confirmed in the present investigation and it would appear that the heavier the contamination of the environment the greater the likelihood of establishment of the staphylococcus in the nose and other carrier sites on the body. The umbilicus is colonised first and most uniformly with large numbers of Staph. aureus. It is doubtful if this seriously affects colonisation of other sites such as the nose since, in an appreciable number of cases, the strain colonising the nares was different from that at the umbilicus, a fact also observed by Gillespie et al (1958). The importance of the umbilicus as a source must not be overrated since, in the majority of cases, it ceases spontaneously to harbour staphylo-:cocci when it separates.

The nasal carrier rate remains high in the first six months of life after which it starts to fall and reaches its lowest level/

level at about two years. Thereafter there is a gradual rise in the carrier rate through childhood and adolescence until the adult rate of 30 % is reached. The rate in young adults remains fairly constant until middle age when a slow fall commences. This is in fairly close agreement with other observations (Williams, 1947).

These findings indicate an initial complete lack of resistance to local colonisation by the staphylococcus in the new-born infant. The sharp drop in the carrier-rate in later infancy and childhood may signify the acquisation of such a resistance. However, this acquired resistance is not permanent, or it is not against all strains of Staph. aureus, for in ensuing years the nares of more and more persons become colonised, until in adult life 30 % are colonised at any one time. It may be that resistance to local colonisation is slowly acquired so that some individuals once again cease to be carriers; meanwhile other persons are becoming carriers as their resistance wanes. In young adults these two processes may just balance one another so resulting in the carrier rate remaining almost constant over a number of years.

Apart from the resistance of the host,other factors would be expected to influence colonisation of the individual. One of these is the amount and frequency of the dose of Staph. aureus to which he is exposed. This may largely explain the difference in the proportion of babies colonised in the home and hospital environments, and also the fact that many families

contain a majority of members who are colonised and others are completely clear. When the dose is very large and the individual repeatedly exposed, lower grades of resistance may be overcome and a larger proportion of persons colonised. Such conditions prevail in the majority of larger hospitals where reservoirs such as the dust contain relatively large numbers of Staph. aureus. Newcomers to the hospital environment frequently acquire the predominant hospital strain. Those persons who were already carriers seem to be more susceptible to colonisation with the hospital organism than those who are not carriers (Clarke,1957).

The second factor is the nature of the colonising strain, but the evidence on this is conflicting. Among the non-hospital population examined there was no evidence that any one particular phage type of Staph. aureus was more liable to colonise the anterior nares than another since the distribution of a large number of different types was fairly uniform .

Within families the tendency appears to be for the established strain, characteristic of the family to colonise other members and maintain itself at the expense of other strains which may be introduced from time to time into the family. High rates of colonisation have been shown among babies and nursing personnel in hospitals and the strains acquired have been those of the hospital environment, comprising a limited number of phage types known to be widespread among carriers, patients with lesions and reservoirs. Thus it may be that these strains have a peculiar ability to colonise healthy individuals as well as infect wounds. When persons who are colonised with hospital type strains of Staph. aureus return to the general non-hospital environment they do not always remain colonised with

these strains for a long period. Among a group of babies who were (Cruickshank,1959) who were born in a maternity hospital during the time of closure of the practitioners' usual nursing home there was indeed evidence of persistence of the hospital type staphylococcus in the families concerned, which strongly suggests that the strain concerned, a type 52/80, had the ability to establish itself at the expense of carrier strains already present. Since the babies born in hospital had had no previous experience of types of staphylococci other than those acquired in hospital, they may be more likely to retain them as their natural colonising flora than adults who acquire similar strains during a stay in the hospital environment. Thus it may be that the main influx of hospital type strains of Staph. aureus into the general community is through the hospital-born baby. Persons whose nares are temporarily contaminated with Staph. aureus are of interest from another point of view. The findings quoted above show that the bacteriophage types isolated from these temporary carriers corresponded to the strains isolated from the environment in which they were living. Thus these carriers are an indication of the degree of contamination of air-borne dust with the staphylococcus and therefore they reflect (indirectly) the degree of contamination of the whole environment since most reservoirs contribute to the dust.

Thus it was common to find considerable numbers of

nose-swab/

nose-swab cultures from nursing personnel in hospital containing small numbers of Staph. aureus but few from persons living in their homes unless there were persons with lesions and carriers in the household. Temporarily contaminated persons also reflect the large number of strains in air-borne dust that cannot be typed by the staphylococcal phages that are at present available.

Persistent and intermittent carriers usually continued to carry the same phage type of staphylococcus during the period they were observed in the same environment. Thus there was no evidence of frequent replacement with different strains under uniform environmental conditions. However, replacement did frequently occur if carriers changed to another environment containing different strains of Staph. aureus. This was most clearly seen in the case of the nurses in the Pre-Training School. Within a matter of a few weeks the majority of these nurses who were already carrying Staph. aureus, had their nasal carrier strain replaced with one peculiar to their new environment. As already discussed, this can be explained on the basis of continu-:ous exposure to a relatively heavily contaminated environment. Medical students examined over a long period both before and after commencing hospital practice showed no similar evidence of replace-:ment of their natural carrier strains, and this was probably due to their relatively short and sporadic contact with the hospital environment.

A limited number of strains of Staph. aureus were isolated from/

from the hospital environment over a number of years though the relative proportions of each strain varied from time to time. Thus the investigation into the spread of Staph. aureus in a maternity unit revealed marked changes in the predominant strains isolated from nurses, babies, patients with lesions and the environment over a 9 month period. At the beginning of the investigation in November, a strain 75/76/77 was predominant in the anterior nares of the nursing staff of the maternity unit, and type 52/52A/80 colonised the majority of the babies. Both strains were recovered from the environment, that recovered from the nurses being present in larger numbers. Breast abscesses contracted during this period, though coming to notice at a later date, were mainly due to strain 52/52A/80. During the early spring, strain 75/76/77 was isolated with increasing frequency both from the babies and the environment, until by April it was the predominant strain. However, it was less frequently isolated from the nurses. Breast abscesses contracted during this period were more frequently due to the strain, type 75/76/77.

A change over in staff is one possible explanation of the change in strains isolated from the nurses though it is not entirely a satisfactory one when the records of individual nurses are examined. It may have been that at some time before this investigation the predominant strain among the nurse carriers was 52/52A/80 and that strain 75/76/77 was introduced by changing of staff and, owing to environmental conditions, was able to displace strain 52/52A80 from other sites. Eventually

strain 75/76/77/

strain 75/76/77 established itself in some babies and spread from them to become the predominant strain.

The strains isolated from the general population and environment outside hospital were of many and diverse phage types. Almost equal numbers of these strains belonged to the main phage groups but within the groups there were a very large number of distinct types. Compared with the strains isolated from the hospital population and its environment there was a very much larger number of distinct types. Most noticeable were the very large number of strains with phage patterns of Group II, which were rarely encountered in hospital.

The outstanding feature about the hospital strains was the preponderance of Group III strains in the earlier years of the investigation. The same phenomenon was observed in hospital environments in the United States, Canada, Australia and the United Kingdom (Barber, 1949; Williams, Rippon and Dowsett, 1953; Rountree, 1953; Fusillo et al, 1954; Knight and Holzer, 1954; Wallmark, 1954), though a few reports concerning hospital outbreaks of infection incriminated strains of Group I (52 and 52A)(Elwood, 1951). During the last few years however, reports in the literature have stressed the importance of Group I strains in outbreaks of hospital infection though most reports do not indicate whether or not the same strains are as widespread among carriers and in the environment. Most recently, strains with patterns of lysis due to phages 80/81, 42B, 52 and 52A have become widespread throughout the world and many reports/

reports ascribe outstanding powers of virulence and colonisation to these strains (Wysham and Kirby, 1957; Timbury, Wilson, Hutchison and Govan, 1958; Hennessey and Miles, 1958; Williams, 1959; Mitchell et al, 1959).

The experiences reported in the foregoing experiments bear out that a change in the preponderance of certain strains of Staph. aureus has occurred in the hospitals in which continued observations were made, but there has been no introduction of new strains. Important in this respect is the fact that phages 79 and 80 (81) were only introduced for routine typing after 1955 and therefore strains isolated before this date were not tested for susceptibility to these phages. None of the strains of Staph. aureus isolated during the present investigation after 1955 were lysed by phage 80 only, though a proportion were lysed by this phage in company with one or more other phages of the same Group (I). Freeze-dried preparations of strains isolated prior to 1955 have been examined and compared by simultaneous typing with strains isolated after 1955 and most of them gave similar or identical patterns of lysis. One must assume therefore that there has been no introduction of strains of the "type 80" comparable to those described elsewhere.

It is our opinion that the Group I strains isolated in recent years in greater numbers in this geographical area are similar to those isolated 5 or more years ago, but at the same time there is no doubt that the relative proportion of these strains/

strains has increased and this increase has been associated with a rise in the number of strains resistant to multiple antibiotics.

More recently there has been a change in the distribution of strains of the main phage groups in the general population so that more are now of Group I and III than was the case 5 to 10 years ago. This again is associated with a change in the antibiotic resistance of strains and stresses the importance of this factor in the epidemiology of staphylococcal infections.

The most obvious explanation for the change of phage types of Staph. aureus among carriers in the general population is the spread of strains from the hospital environment. This is supported by the fact that the Group I and Group III strains isolated from the general population have phage patterns similar to those that have been isolated in hospital, and moreover, in many cases, these strains were isolated from persons who had been in hospital or were in close contact with persons who had been in hospital. It is also apparent that patients may be infected in hospital with hospital strains of Staph. aureus and leave with the infection still active or the organism colonising such sites as the anterior nares (Williams et al, 1959); those who have had surgical interference may have contracted cross-infection which developes after discharge from hospital; infections in the new-born and their mothers often appear after discharge from the maternity hospital and are most frequently due to hospital strains (Nelson, 1952; Isbister, Durie, Rountree and Freeman, 1954; Brown and Berry, 1956; Ravenholt and LaVeck 1956; Wysham et al, 1957;

Cook, Parrish and Shooter, 1958); patients in medical wards with bronchitis may suffer staphylococcal superinfection of their respiratory tracts (Mayard Oswald, 1956; Oswald, Shooter and Curwen, 1958; Walker et al, 1958). All of these persons may bring their hospital acquired staphylococcus to the home environment, either in a resolving lesion or colonising some other site such as the anterior nares. A proportion of these strains undoubtedly maintain themselves for some time in the home environment as has been clearly shown by a group of babies born in a maternity hospital and who were discharged colonised or infected by a hospital type strain, 52/52A/80 (Cruickshank, 1959). One hundred episodes of staphylococcal infection due to this strain occurred in 15 families over a period of 18 months and at the end of this period members of several of the families were still colonised by the strain.

It is not entirely clear whether staphylococcal infections are more numerous now than they were before antibiotics became available because there are few, if any, objective accounts of their incidence available for comparison.

It is unlikely that there has been any material change in either the morbidity of staphylococcal infections in general practice during the past 20 years. Most attention has been paid to staphylococcal infections occurring in hospitals and some observers have maintained that, in this case, there has been an increase (Howe, 1954;1956). It may be that in most instances this is relative, due in part to the exclusion of the streptococci/

streptococci as infective agents, and also to the fact that more attention has been focussed on minor lesions.

What has undeniably changed since the advent of anti-:biotics is the susceptibility of strains of Staph. aureus to these agents, and this itself has played a large part in drawing attention to staphylococcal lesions,particularly so since the resistance of strains interferes with the prevention and treat-:ment of infections with antibiotics. The increase in the proportion of antibiotic-resistant strains of Staph. aureus for many years a feature of hospital environments is now becoming apparent in the general population. This is a most disturbing epidemiological feature of staphylococcal infections at the present time.

Antibiotic-resistant strains were observed in patients receiving penicillin shortly after the introduction of the antibiotic. These organisms were penicillinase producing staphylococci, as have been all other naturally occurring penicillin-resistant strains of clinical importance (Barber, 1947; Spink and Ferris, 1947; Chandler, 1951; Finland and Haight, 1953). This resistance has not precluded successful treatment in a proportion of cases infected with such strains since large doses of penicillin are frequently sufficient to control the growth of relatively small numbers of the organism in the tissues which are not producing a large amount of the antibiotic-destroying enzyme (Bowie, 1953). It is quite apparent from in vitro sensitivity tests that these strains vary greatly in their resistance/

resistance to penicillin, though the results obtained depend upon the inoculum size and the phase of growth (Barber Parker, 1946).

The proportion of penicillinase-producing strains increased rapidly in hospitals during the years when penicillin was the only antibiotic used systemically on a large scale, so that by the time streptomycin was introduced more than one-half of all strains isolated were resistant to penicillin. The introduction of streptomycin and subsequently chloramphenicol and the teracyclines, was followed by an increase in the proportion of strains of Staph. aureus resistant to these antibiotics. The outstanding features of these strains as examined in the present investigation have been:-

1. That all of the antibiotic-resistant strains have been penicillinase producers. Not one strain resistant to strepto-:mycin, chloramphenicol or the teracyclines has been isolated and found not to produce penicillinase. Thus all penicillin-sensitive strains have been fully sensitive to the other anti-:biotics.

The detection of penicillin susceptibility of strains of Staph. aureus has therefore clinical usefulness and makes the testing for susceptibility to other antibiotics unnecessary.

In the laboratory a number of strains have been trained to grow in increasing concentrations of the different antibiotics and variants obtained were resistant to individual antibiotics and in many cases did not produce penicillinase. These antibiotic-resistant/

antibiotic-resistant variants included "in vitro" penicillin-resistant cultures growing in 10 units per ml. of penicillin and which did not produce penicillinase. The growth characters of these penicillin-resistant cultures were quite different from the naturally occurring penicillin-resistant strains, and as they are rarely isolated from patients or carriers they would appear to have no clinical importance. The strains of staphylococci induced to become resistant to streptomycin, chloramphenicol and the tetracyclines did not show markedly different growth characters from naturally occurring resistant strains, apart from the fact that they were penicillin-sensitive.

2. The appearance of antibiotic-resistant strains is definitely related to the introduction of the particular antibiotic, and the proportion of strains found to be resistant is related to the amount of antibiotic used. In this investigation it was shown that a much greater proportion of carriers of penicillin-resistant strains had had previous experience of penicillin than was the case with carriers of penicillin-sensitive strains. Also the majority of the carriers of strains resistant to antibiotics other than penicillin who were observed in the general population, had had therapeutic experience of these antibiotics. In hospital it was also observed that the pro-:portion of streptomycin-resistant strains isolated from carriers and patients with lesions was proportional to the amount of this antibiotic used.

3. The antibiotic-resistant strains are limited in number as shown/

shown by the range of phage types involved. As has been already described, the majority of antibiotic-resistant strains of staphylococci occurring both in hospital and in the general population belonged to phage Group III. More recently strains of phage Group I have been isolated with greater frequency in hospital and to a lesser extent in the general population which were resistant to several antibiotics. Strains of Group II resistant to antibiotics other than penicillin were rarely isolated.

Spontaneous mutation is generally accepted as giving rise to antibiotic resistant cells in a given bacterial populat-:ion but it is probable that only a limited number of strains of Staph. aureus are capable of giving rise to such mutants. These mutants become apparent only if there are factors in the environment which are capable of selecting them by favouring their growth at the expense of the antibiotic sensitive populat-:ion. The most obvious selective factor is the antibiotic itself and the facts given above suggest that the antibiotics used therapeutically have been of considerable importance as selective factors.

Inside a closed community such as a hospital the selection process has been more powerful and effective in eliminating antibiotic-sensitive staphylococci. Not only have the sensitive cells in populations of strains capable of giving rise to resistant mutants been suppressed, but there has been virtual elimination of the entire populations of strains that do not give rise to such mutants. The overall effect of this/

this has been to reduce the number of different strains of Staph. aureus in the hospital environment leaving only those that are antibiotic-resistant. From time to time antibiotic sensitive strains are introduced by new personnel or patients but they will also be rapidly eliminated unless they give rise to mutants capable of surviving in the hospital environment. Initially all of these resistant populations were penicillin-resistant because of their ability to produce penicillinase. Some of these surviving penicillin-resistant strains have had mutants resistant to streptomycin, chloramphenicol, tetracyclines and erythromycin, and these have been selected in turn as respective antibiotics were introduced, and have provided the population of Staph. aureus resistant to multiple antibiotics which are commonly encountered in hospitals to-day. This mechanism would be expected to give rise to a decreasing number of distinct strains of Staph. aureus as successive antibiotics were introduced since only a limited number of strains would be capable of giving rise to mutants resistant to many antibiotics. The evidence is compatible with this and the marked predominance of strains such as 52, 52A, 52/52A/80 and 80/81 may reflect such a selective process. Strains of these and related phage patterns isolated in this present investigation were notable for their marked variability in resistance to many of the antibiotics. This wide range of adaptability by virtue of mutants, capable of surviving in the presence of most, if not all, of the antibiotics in use, may be the chief reason why strains of these patterns have become so widespread and predominant in hospital environments.

The/

The adaptability to antibiotics of a Group III strain, type 75/77, first isolated in Leith Hospital from carriers and patients' lesions in 1954, has probably allowed its survival and overwhelming predominance in the Eastern General Hospital, an associated hospital, in 1958. This single strain was isolated from more than two-thirds of carriers and lesions as well as in large numbers from reservoirs in the environment during the present investigation. The most frequently isolated of other strains was 52/52A/80. The antibiotic-resistance of both strains varied considerably in different isolations, ranging from resistance to penicillin alone to resistance to penicillin, streptomycin, chloramphenicol and the tetracyclines. Strain 75/77 however, in 15 % of the cultures isolated, was also erythromycin resistant, and it was significant that erythromycin was being used as a routine therapeutic agent at this time in the hospital. No cultures of strain 52/52A/80, resistant to erythromycin, were isolated. This suggests that strain 75/77 predominated because of its ability to grow in the presence of erythromycin which the other strains were not capable of doing.

The Spread of Antibiotic-resistant Strains

The mechanism by which antibiotic-resistant strains arise may be in dispute. What is of more immediate importance is the way they are spread through the community. Whether the resistant mutant arises by mutation or adaptation its further selection and maintenance can be readily explained in the person who is receiving therapeutic antibiotic, for in such cases the environment in which the organism is growing has become highly favourable and selective. Patients who are already receiving antibiotic for prophylactic purposes, or for some non-staphylococcal infection will also afford favourable sites for the successful establishment of antibiotic-resistant strains of staphylococci which contaminate by cross-infection. Staphylococcal carriers receiving antibiotic may have their antibiotic-sensitive carrier strain eliminated and replaced by an antibiotic-resistant. The resistant strain may have been present in the minority and previously unrecognised, arisen as a mutant, or been inspired in contaminated dust. This is /

is borne out by the results of the investigation of the relation-:ship of antibiotic therapy to the carriage of antibiotic-resistant organisms.

However it is an outstanding feature, confirmed by the present observations, that antibiotic-resistant strains are predominant in the hospital environment but very much less numerous in the home environment. In the hospital environment the resistant strains are ubiquitous and are found in patients with lesions and in staff carriers who have never been treated with the antibiotic. The distribution of antibiotic-resistant strains in carriers and the environment was found to be very similar in another situation far removed from hospital, namely in a factory where there were no patients receiving therapeutic antibiotic but where penicillin was being handled on a large scale. The amounts of penicillin recovered from the air of parts of the factory where penicillin was being dispensed was very great, exceeding 1 unit per cub. foot. The exposure of petri-dishes containing nutrient agar seeded with penicillin-sensitive organisms to settling dust for half-an-hour was sufficient to completely inhibit growth of the organism. The antibiotic could also be recovered from the lining of the nasal vestibules of workers in this part of the factory indicating that quantities were inhaled with air-borne dust. This is most important since the nose is one of the chief sites of growth of the staphylococcus in carriers and therefore the site at which the antibiotic is likely to have its most marked effect in selecting/

selecting resistant mutants and favouring their growth. This was confirmed by observing that all of the strains of staphylo-:cocci isolated from the nares of workers in this factory were penicillinase-producing strains.

Penicillin-sensitive staphylococci present in the reserv-:oirs of such environment are similarly affected because they come into contact with penicillin which is discontinuously dispersed in the form of small particles, or agglomerates of particles, some of which are airborne, and others which settle on surfaces. Dust particles may incorporate these antibiotic particles and contaminating micro-organisms on the particles will come into contact with concentrations of penicillin which are locally very high. Thus it is likely that only penicillin-resistant cells will survive in such an environment and this was confirmed since only penicillinase-producing staphylococci were recovered from air-borne dust and the surfaces of fomites that were sampled.

The air and dust-borne antibiotic has more widespread effects because it can be widely dispersed, though at the same time diluted. Thus penicillin-contaminated air and dust was found in other parts of the factory. Staff working in such an atmosphere will inhale the antibiotic and pick it up on their hands from contaminated objects and transfer some of it to their noses and mouths. Thus penicillin-resistant strains also predominated in these persons who were not actually working with the antibiotic.

It is clear from the present observations that penicillin

can/

can be recovered from the hospital environment in the same manner as from the factory. The antibiotic was found in highest concentration at the sites where it was being used. In the dispensaries, wards and out-patient departments of the particular hospital examined, the handling of phials and syringes containing penicillin were among the more important sources. Leakage from the phials during puncturing with the syringe, the clearing of air and froth from syringes and needles before injection, and spraying when washing out the syringe all commonly occur during the process of injecting a patient with a dose of penicillin. Other sources,as obvious, are the insufflation of wounds with antibiotic, and the use of topical preparations such as ear and eye drops. Other sources are not so obvious such as the spilling of urine and the expectoration of sputum containing antibiotic from a treated patient. These secretions may contain up to several thousand units of antibiotic per ml. and after evaporation particles contaminated with antibiotic will be dispersed.

Air may therefore be contaminated in two ways, directly by droplet nuclei, which are the dried residue of droplets atomised from syringes and the like, and indirectly by the raising of dried spilled penicillin in dust. In this way the hospital environment can readily acquire penicillin and other antibiotics, and the effect will be cumulative since destruction of the dried antibiotic in dust is slow, and redistribution continually occurs through disturbance of settled dust and the handling of contaminated articles. Appreciable amounts of

penicillin have been shown to gain access to the nares of persons working in the hospital and without doubt this will inhibit the growth of penicillin-sensitive strains of Staph. aureus and favour the growth of resistant strains. Persons in such an environment will be continually exposed to re-contamination with staphylococci from the environment, and the great majority of these will be penicillinase-producing strains which will find it easy to establish themselves and colonise the nares of larger numbers of persons in hospital than in other environments which do not contain antibiotic, and where the nares of most carriers are still colonised with penicillin-sensitive strains of Staph. aureus.

Thus the predominance of penicillin-resistant Staph. aureus in carriers in a closed community such as a hospital, is explained as also is the fact that the longer the period of exposure to the hospital environment, the greater is the chance of becoming colonised with antibiotic-resistant organisms (Barber, Hayhoe and Whitehead, 1949; Cairns and Summers, 1950; Rountree and Wallmark, 1953: Barber, 1951; Vogelsang and Boe, 1952; Lepper et al, 1953; Hutchison, Green and Grimson, 1957). The results obtained in the penicillin factory, where persons exposed to environmental penicillin but not to penicillin-treated patients, had as high a resistance rate of Staph. aureus as hospital personnel, confirm that environmental penicillin is an important factor which determines widespread colonisation of hospital carriers with antibiotic-resistant strains of Staph. aureus.

The number of carriers among the general population who harbour penicillin-resistant strains of staphylococci is relative-
:ly/

relatively small. These persons are likely to be little affected by environmental penicillin because there is little chance of accumulation of the antibiotic in their homes and less chance of contamination with penicillin-resistant strains of staphylo-:cocci. However it is of significance that penicillin was recovered in small quantities from two households in which penicillin had been used for therapy, and so it is possible that in a limited number of houses antibiotic accumulation could occur after prolonged usage. The chances of this occurring should become less as it is now more unusual for the physician or nurse to dispense antibiotic in the home as the use of ready packed oral preparations is becoming more common. There are however other sources of antibiotic which persons may come into direct contact with and which may also contaminate the environment. Antibiotics are being used on an increasing scale as dietary supplements for animals. This may have the effect of selecting and favouring the growth of antibiotic-resistant flora such as resistant Staph. aureus in these animals which would form a focus of these strains for cross-infection to man. Antibiotics are being used on an increasing scale to preserve poultry, beef and fish for human consumption (Wrenshall, 1956). The treatment of infections in farm animals with antibiotics is extensive, for example the treatment of mastitis in cows leads to large quantities of the antibiotic passing into milk (Storrs and Wiatt-Brown, 1954; Berridge, 1956; Jour. Amer.Med. Asso. 1957; Wear, 1958). By these procedures amounts of antibiotic must reach the consumer in food, for not all the antibiotic is destroyed in storage and cooking, and this may assist in favouring the

growth/

growth of antibiotic-resistant micro-organisms, including Staph. aureus, in man. In addition there is the danger of immunological sensitisation of human tissues to these antibiotics by repeated contact. This is especially important in the cases of penicillin, an antibiotic which is more avidly bound to plasma protein than are other antibiotics.

Restriction in the use of antibiotics and avoidance of contamination of the environment might therefore be expected to reduce colonisation of carriers with antibiotic-resistant strains and thereby reduce spread to patients and a consequent decrease in the number of infections with antibiotic-resistant Staph. aureus.

The widespread, voluntary restriction in the use of chloramphenicol after 1951 did have such an effect on chloramphen-:icol-resistant strains of Staph. aureus (Kirby et al.,1953) and restriction in the use of streptomycin (Needham and Nichols, 1953) and of erythromycin (Lepper et al., 1953) have been followed by a reduction in the incidence of strains resistant to these antibiotics.

For this reason it has been frequently suggested that one or other of the antibiotics in general use be severely restricted for a fixed period so that strains of staphylococci which are resistant to it amy disappear, and others have suggested the rotational use of the major antibiotics. Locally, in Edinburgh, the use of erythromycin has been severely restricted on a voluntary basis, and erythromycin-resistant strains of Staph. aureus are rarely encountered, except in one hospital where/

where the antibiotic is used to treat staphylococcal infections. The restriction of penicillin to reduce the number of penicillin-resistant strains which are the most numerous of antibiotic-resistant strains of Staph. aureus has been often suggested and occasionally attempted. Apart from the enormous administrative difficulties in carrying out such a scheme it is unlikely that it would have the desired effect for the results of the present investigation show that all antibiotic-resistant strains are penicillin-resistant and therefore the mere restriction of penicillin with the continued use of the other antibiotics would still favour and select the growth of strains that were penicillin-resistant. Thus it would appear that the most hopeful policy is to curtail the use of all antibiotics to the minimum compatible with efficient treatment. Antibiotics are still used indiscriminately in the treatment of many infections and frequently as a prophylactic, and even as an aid to diagnosis. Wherever possible antibiotics should be used only in cases after bacteriological diagnosis has been made and sensitivity tests have indicated the most suitable antibiotic or combination of antibiotics to use. after the most suitable antibiotic has been selected treatment should be carried out for as short a time as possible.

The administration of antibiotics should be carried out so as to minimise the chance of contamination of the environment. Where antibiotic solutions or suspensions require to be prepared for parenteral injection, care should be taken not to spill or spray antibiotic into the environment. Ready prepared, single dose/

dose ampoules fitting a special syringe would be preferable. The loading and washing out of syringes from ampoules may be carried out in a cabinet fitted with exhaust ventilation and whose floor is covered in material soaked in inactivating agent. Such a cabinet has been in use in one department in the Royal Infirmary and 2 - 5 % sodium carbonate has been found a suitable agent which destroys penicillin rapidly. The walls of the cabinet are washed daily with carbonate solution and the floor is laid with 4-ply 'Cellosene' wadding soaked in carbonate solution which is renewed each day. Syringes after use are washed out in 5 % sod. carbonate and left in the solution for at least an hour before repeated washing in distilled water prior to sterilis-:ation.

Antibiotics which are administered systemically should not be used topically where there is a chance that they may contaminate the environment. There are numerous antibiotics and other antibacterial agents available which cannot be used systemically and which are suitable for topical application.

The use of antibiotics for the preservation of food and the marketing of milk containing titratable antibiotic require serious investigation and curtailment if it is found that a significant amount of antibiotic reaches the consumer.

The Dissemination of Staph. aureus

Carriers of Staph. aureus are important as a source of the organism because they disseminate it to the environment. A more obvious source of the organism available for dispersion to the environment/

environment is the patient suffering from a lesion and many investigators believe these persons to be more important as sources than carriers. Thus outbreaks of staphylococcal infection in hospital have been traced to a member of the staff, or to a patient suffering from a discharging lesion due to a strain of the same type as that producing the subsequent infection (Shooter, Griffiths, Cook and Williams, 1957; Williams, Talbot and Maughan, 1957; Mitchell et al., 1959).

The numbers of staphylococci recovered from nose swab cultures from carriers varies considerably, and it might be expect-:ed that individuals yielding a heavy surface growth of Staph. aureus would have the largest number of organisms available for dissemination to the environment. The findings of Hare and Thomas (1956) and Hare and Ridley (1958) suggest that this is not always the case in either nasal or perineal carriers.

Since, in some outbreaks of staphylococcal infection due to a single strain of Staph. aureus, only a few carriers harbour-:ing the same strain may be observed, the concept of 'dangerous' and 'safe' carriers has arisen. Thus carriers of a strain such as type 80, known to occur in numerous infections, would be regard-:ed as potentially more dangerous in the hospital environment than carriers of other phage types not frequently occurring in cases of infection. However as most of the phage types that have been isolated from carriers in this investigation have also been isolated from infections, the differentiation between 'dangerous' and/

and 'safe' carriers has not been applicable and would not appear to serve any useful purpose. All coagulase positive strains must be regarded as potentially pathogenic.

The nasal carriers examined dispersed Staph. aureus in variable numbers not closely related to the numbers isolated from nose swab cultures of the corresponding carriers. These staphylococci were spread from the nose to other parts of the carrier's body. Very few were discharged into the air during breathing. The saliva rarely contained Staph; aureus in the adults examined which is in contrast to the fairly large numbers recovered from the saliva of a significant proportion of the new-born babies examined. Staph. aureus pass back from the anterior nares to the naso-pharyngeal wall in many carriers and the majority are probably swallowed. The saliva destroys the remainder by means of the inhibitory activity of lysozyme and other substances which it contains. The result is that negligible numbers of Staph. aureus are dispersed by speaking, coughing or sneezing as the experiments reported confirmed. An important point arising from this observation is that nose and face masks will have little or no effect on the dissemination of staphylococci from carriers and this has recently been confirmed in practice by Maccabe and Forfar (1958). This does not mean that masks are ineffective in preventing the dissemination of other organisms of the upper respiratory tract such as strepto-:cocci.

Anything which touches the nose of a nasal carrier will pick/

pick up large numbers of Staph. aureus. Thus the handkerchief is very heavily impregnated with the staphylococcus which is transferred from the handkerchief to the clothes and hands as well as to the air and other objects with which it comes into contact. The hands of most individuals touch the nasal orifice frequently; the hands therefore pick up large numbers of staphylococci which are transferred to other parts of the body surface and to the clothes. It is also probable that staphylococci spread directly from the nostrils over the surface of the skin in sweat and sebaceous secretions. In these ways large numbers of Staph. aureus are distributed over the surface of a carrier's body; his clothes become heavily impregnated so that all body movements, the use of his handkerchief and the handling of objects involves the dissemination of cocci into the air or onto the surface of objects. Persons who carry the organism in other sites distribute it in a similar manner though there are differences in the degree of contamination of parts of the body or clothes; thus the perineal carrier does not have his handkerchief so heavily contaminated as that of the nasal carrier, and one would expect that their undergarments would be more heavily contaminated than those of nasal carriers though this has not been tested.

Dissemination of Staph. aureus from carriers to the environment is thus by two main mechanisms. (a) by dispersal into the air as discrete cocci, contaminated dust particles, and (b) by contamination of surfaces by the hands. In the present experiments on nasal carriers, the most important route of dispersal/

dispersal, as measured by the number of organisms liberated, was by the handkerchief, and the least by speaking, coughing or sneezing. One use of the handkerchief by a carrier liberated the same number of organisms as 10 minutes of minor body movements or 100 hours of normal breathing activity.

The number of Staph. aureus transferred by contact with the hands of carriers varied considerably from individual to individual and was of course influenced by washing the hands prior to sampling. Staphylococci were transferred in appreci-:able numbers to both wet and dry surfaces in tests simulating the touching of surfaces in everyday life, and it was proved that staphylococci were transferred during the act of handshaking.

A most important feature of the tests showing dispersal of staphylococci into the air was the demonstration of numbers of organisms for periods up to 3 hours after the carrier had left the test chamber (Fig. 14). Thus a carrier walking into a room will leave Staph. aureus air-borne in the dust for hours, and after settling, the dust will remain contaminated as long as the cocci remain viable which may be for a period of several months.

It was proved conclusively that non-carriers did not disseminate Staph. aureus in any of these ways, though small numbers may be present on their hands or clothing as a result of cross-contamination from another source or reservoir.

Persons suffering from superficial staphylococcal lesions were examined to find out if they dispersed comparable numbers of staphylococci/

staphylococci to the environment. The patient in hospital, with a grossly infected and suppurating wound open to the surface, must disseminate large numbers of Staph. aureus to his bedding, his dressings, his hands, toilet requisites and so on (Colbeck, 1957). It was not possible to examine such a patient under conditions comparable to those used for the carriers, but ambulant patients with 'closed' and 'open' staphylococcal lesions such as boils and styes, were examined and it was found that the numbers they disseminated were negligible if the lesion was 'closed' unless they were nasal carriers; the numbers were comparable to those disseminated by carriers if the lesions were discharging. This is not altogether surprising since nose swabs from a carrier often yield a heavier growth of Staph. aureus on culture media than similar swabs which have sampled the exudate from staphylo-:coccal lesions. The patient with a'closed' lesion does not disperse the staphylococcus from the lesion. This was obvious in the series of examinations carried out on persons in general practice and suffering from superficial infections, as only 11 % yielded Staph. aureus from skin around the lesion.

It must be concluded that the majority of persistent carriers are as prolific and dangerous a source of staphylococci as persons who have staphylococcal lesions. Since carriers are very much more numerous than persons with lesions they are, numerically at least, more important as a source of the organism. Overall they may not be as dangerous if there is a significant difference in the virulence of strains of Staph. aureus since the strains derived from a patient with a lesion are likely to be always virulent whilst this would not be the case with many carrier/

carrier strains.

Examination of various environments for the presence of Staph. aureus in air-borne dust and on the surfaces of fomites showed that the numbers were related to the presence of sources such as carriers and patients with lesions. Thus in private houses many more staphylococci were recovered from those where known sources lived than from those free of such sources. Similarly in laboratories the highest yield came from those specifically handling staphylococcal cultures.

The two methods used for sampling Staph. aureus in air-borne dust measure rather different things and cannot be directly equated. The sieve sampler collected particles which were suspended in the air and most of these would be particles less than 25 micra in size and few particles could contain more than 2 or 3 cocci. Settling plates on the other hand collect a greater proportion of larger particles which can only remain suspended for a relatively short time in the absence of repeated agitation of the air. Many of these particles will be large enough to contain hundreds or more individual cocci. By either method however, each contaminated particle will give rise to one colony. The larger particles provide bigger inocula and may therefore be more important from the point of view of initiating infection when they alight on exposed surfaces such as an open wound.

It is of interest to note that the percentage of particles contaminated/

contaminated with Staph. aureus settling on plates was usually considerably higher than the number of similarly contaminated particles recovered from sieve sampler plates. This may mean that the dust is contaminated with a large number of particles which contain agglomerates of staphylococci.

Inside hospital much larger numbers of Staph. aureus were recovered from the environment than outside, with the exception of homes where there lived persons with recurrent staphylococcal lesions. The most heavily contaminated parts of the hospital were the wards, especially the maternity wards, and the operating theatres. At these sites more than 1 % of all organisms recovered from the air-borne dust were Staph. aureus. The proportion of staphylococci recovered on the settling plates did not always correspond to that on the sampler plates. This was especially so in the operating theatres where the total counts of bacteria recovered were lower than at other sites in the hospital but the proportion of Staph. aureus was higher than on the sampler plates. On one occasion 10 % of all settling particles contamin-:ated with bacteria contained Staph. aureus and this may be one reason why wounds are frequently contaminated in theatres.

The sampling of surfaces of fomites was carried out by swabbing, by pressing the surface on to agar plates or, in the case of bedding and drapes, by flapping or brushing over the surface of a plate. A crudely standardised swabbing technique for each site was found to be valuable in giving an indication of the/

the degree of contamination.

The evidence shows that strains of Staph. aureus isolated from patients' lesions, carriers and reservoirs in the hospital environment differ in their susceptibility to antibiotics and bacteriophages from those isolated outside of hospital. In both situations however there is a close correspondence between the strains isolated from carriers and lesions in patients.

Outside of hospital, among general practice patients, the carrier may infect himself. As has been discussed the staphylococcus spreads over his body surface and impregnates his clothing. It is not surprising therefore to find that the great majority of superficial staphylococcal lesions in these patients occur at sites nearest the nares or at sites within easy access of the hands or which are rubbed or chaffed by the clothes. Repeated inoculation of hair follicles and sweat glands and small breaks in the skin must occur. Some factor other than the organism or its toxic products must be responsible for the successful initiation of infection since the minimum number of cocci required to produce a small pus forming lesion when inoculated into the skin is of the order of a million or more (Elek, 1959), and this is not greatly reduced by the simultaneous inoculation of preformed toxin. The presence of foreign bodies has been shown to definitely reduce the dose required (Elek and Conen, 1957) and inspissated sebum blocking a gland orifice and trapping staphylococci may act in this manner. The possibility of hypersensitivity to the staphylococcus or its products must also/

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also be borne in mind (1958), and in favour of this is the observation that lesions may abort when the organism is suppressed in the nares.

The carriers are colonised with Staph. aureus before contracting lesions and do not become carriers after having had a lesion, though a few non-carriers who have had a lesion may become carriers. It is probable that many persons are carriers for years before having their first lesion. It may be that an element of hypersensitivity develops to make these persons react to the presence of the staphylococcus.

At least 5 % of the general population examined have one or more staphylococcal lesion each year and about one-half of these suffer from recurrent boils or styes and similar lesions. The practice examined was non-industrial and the majority of the patients came from the lower middle-class. It is probable that the number who suffer from staphylococcal lesions differs in other social grades and in industrial practices a higher proportion of infect-:ions will be infected injuries. The number of Staph. aureus present in the environment is usually small and cross-infection is therefore less important in the home than in the hospital environ-:ment. It occurs when an abrasion is infected and in a small number of non-carriers boils and other non-traumatic staphylococcal lesions occur, usually by contact with carriers in the family.

Cross-infection on the other hand is important in the hospital environment since the strains of Staph; aureus producing infections/

infections in patients are nearly always those found in hospital staff carriers and reservoirs in the environment. The majority of the lesions are superficial wounds which have been exposed at some stage to contamination with the organism. Others involve the establishment of the hospital staphylococcus on surfaces of the gut or respiratory tract because of abnormal circumstances in the host such as antibiotic therapy, established infection with micro-organisms other than staphylococci, or trauma. Thus staphylococcal bronchopneumonia, staphylococcal enteritis and cystitis may be due to hospital cross-infection in some cases. Only small numbers of cocci may be required to establish infection under the conditions pertaining in these patients where the natural defence mechanisms have been breached, as occurs in the wound, or impaired in the patient who is already ill. This was apparent in a number of patients with chronic bronchitis who were recently examined (Murdoch et al., 1959). Twelve out of 75 of these patients were found to have their upper respiratory tracts colonised with hospital type staphylococci after six months observation. These patients were treated at home and the only possible spurce of contamination was a waiting room in the hospital out-patient department where they were examined each fortnight. The dust and fomites in this room were contaminated with hospital type Staph. aureus in relatively small numbers so that a few would be inhaled by these patients from time to time.

An important group of lesions which occur in hospital are those occurring in hospital staff for they form an important source/

source to contaminate the hospital environment. Clinically the lesions in these persons are similar to those found in the general population, and in many cases the epidemiology is probably similar as the causative organism is found in the anterior nares. However since most of these strains are hospital types and similar to those in patients and the hospital environment, it is difficult to be certain about their epidemiology. If the infections among the nurses are separated into two groups, one comprising boils and styes and other infections without any traumatic history, and the other comprising lesions such as paronychia, and/ abrasions which are associated with an element of trauma, there is found to be a significant difference in the nasal carrier rate between the groups. In the first, the carrier rate is over 90 % and in 70 % the same organism occurred in both nares and lesion suggesting that auto-infection is common. In the second group the carrier rate was less than 50 % and the same organism occurred in lesion and nose in only 30 %, so that cross-infection was more common.

Prevention of Staphylococcal Infection

A knowledge of the epidemiology of staphylococcal infections occurring both inside and outside hospital indicates that several different methods of prevention may be successful in preventing the spread of the causative organism and its establish-:ment in the tissues of susceptible hosts.

The factors which determine individual susceptibility to Staph. aureus/

Staph. aureus are unknown so that the institution of measures to increase host resistance to infection are difficult. Diseases such as diabetes mellitus and infection with the influenza virus seem to lower resistance so that control of these conditions should decrease the likelihood of infection. The effects of poor hygiene and nutrition are ill-defined, but the presence of trauma such as wounds or burns and their inclusion of foreign bodies or tight surgical sutures increase susceptibility (Elek and Conen, 1957). So far little has been deduced of the specific cellular and humoural mechanisms of resistance to staphylococci and only equivocal success has resulted from active and passive immunisation with bacterial vaccines, toxoids and antitoxins (Wise, 1958; Burnett et al., 1958).

As Staph. aureus is widely distributed in the environment, and sources such as carriers numerous, it is difficult, if not impossible to prevent these organisms contaminating susceptible individuals such as young infants, patients on antibiotic therapy, those with virus infections of the respiratory tract and wounds. In certain cases access of contaminating micro-organisms may be prevented by covering a part of the patient, for example a wound, and this has been practised in the treatment of burns in hospital (Colebrook, 1950). It is most important to avoid mixing infected and uninfected patients both in the wards and on operating lists. An attempt may be made to isolate susceptible patients, especially those suffering from predisposing disease such as agranulocytosis, influenza or bronchitis, but on a large scale/

scale this is a difficult and expensive measure.

More attention has been paid to the control of circulat-:ion and dispersal of staphylococci in the hospital environment. It has been recommended that 'clean' and 'dirty' work be carried out in widely separated parts of the hospital so that the environment of uninfected cases is not heavily contaminated (Nuffield Hospitals Report, 1956). Many hospitals lack proper dressing rooms where there are facilities for attending to patients' wounds, avoiding their contamination by Staph. aureus and reducing the chance of infected wounds dispersing pathogens to the environment. To this end great care must be taken in the disposal of soiled dressings for when saturated with pyogenic exudate many millions of micro-organisms may be dispersed during movement and handling. They are best handled gently and immediately places in impervious paper bags which can be inciner-:ated.

Staphylococci are disseminated from the clothing and footwear of many persons. This may be minimised by reducing the amount of traffic in hospital, and in special situations such as surgical theatres no person ought to enter before doffing all outside clothing, washing and re-clothing in sterile garments including footwear (Wise, 1958). Hospital staff should be encouraged to change into clean clothing frequently when on duty and pay great attention to toilet of the hands.

The dust of the environment is heavily contaminated with Staph. aureus which is spread from other reservoirs and sources/

sources through the medium of the dust. Thus any measures such as the oiling of blankets and floors with spindle oil (M.R.C., 1951), the use of damp dusting and vacuum cleaning which trap dust and prevent its circulation will reduce the chances of contamination with micro-organisms.

Decontamination of the environment and fomites will reduce the number of micro-organisms, including Staph. aureus which have access to new hosts, especially if the rate of decontamination exceeds the rate of supply of fresh organisms. Air-borne bacteria may be destroyed on a limited scale by means of ultra-violet radiation and this method has some application in dressing-rooms and surgical theatres where it may be used in conjunction with air-filtration to remove bacterial laden dust.

Probably the most efficient method of reducing bacterial contamination of air is to allow adequate ventilation and sunlight.

Blankets, mattresses and pillows and other bedding are usually heavily contaminated with bacteria, a proportion of which are Staph. aureus, and these are thrown into the air in large numbers whenever beds and bedclothes are disturbed in any way. Cotton and linen sheets may be disinfected by boiling and launder-:ing but this is not possible with woollen blankets which shrink. Woollen blankets may be laundered at 100°F and disinfected with a cold rinse containing quaternary ammonium compounds (Gillespie and Robinson, 1959) which kills nearly all vegetative bacteria. Formalin disinfection can be used but requires expensive equipment and/

and the separate handling of each blanket. Cotton and synthetic fibre blankets do not contribute so much dust to the environment and may be disinfected by boiling (Blowers,Potter and Wallace,1957; Thomas and Liddell,1958).

Bedding becomes rapidly contaminated with Staph. aureus from carriers and patients qith lesions (Colbeck,1957) and it is important to change bed-linen frequently. Mattresses and pillows share in this contamination but their decontamination is more difficult. It is thus better to prevent impregnation of such articles by enclosing them in impervious rubber or plastic jackets which can be regularly washed with antiseptic solutions.

The surfaces of wash-basins and baths become heavily contaminated with staphylococci and other micro-organisms (Boycott, 195) after use. Disinfection of their surfaces can be carried out by thorough scrubbing and washing with solutions such as sodium hypochlorite. There is probably a place for more use of disinfectants in the environment than at present, for example in treating contaminated surfaces and in mats for wiping the feet (Burnett et al.,1958). The development of aerosols to disinfect airborne dust warrant further investigation (Challinor,1943)

Less attention ahs been paid to controlling the staphylococcus at its source. The lesions in patients are obvious and these persons may be isolated to prevent contamination of reservoirs or new hosts. Members of hospital staffs suffering from staphylococcal lesions should not remain on duty. Carriers of the staphylococcus are much more numerous though not so obvious as persons with lesions. It is quite impracticable to isolate

carriers or keep them off duty because of their large number and it is clear from the present investigation and that of others (Maccabe and Forfar,1958) that gowning and masking are quite inadequate to prevent the dissemination of their staphylococci.

Since Staph.aureus grows only in defined areas of the body surface of carriers the application of inhibitory substances to prevent their growth and spread can be successful. This has been proved in a number of carriers by measuring a reduction in their dissemination of Staph. aureus after the topical administration of anti-staphylococcal substances. If an antibacterial agent is chosen which is specifically active against the carrier strain the dispersal of the staphylococcus can be reduced to a negligible amount in more than 90% of carriers as shown by nose swab cultures becoming negative for Staph.aureus. It is sufficient to administer the antibacterial substance intermittently, for example one week in three, to achieve reduction in 75% of carriers.

The practical efficiency of this method for limiting dispersal of staphylococci from source was shown by measuring the reduction of auto-infection in carriers in the home environment following treatment, and of cross-infection in patients in hospital following the treatment of carriers among the staff. A similar experiment in a maternity unit resulted in a suggestive drop in the number of environmental staphylococci.

The antibiotics in general use have proved to be the most potent anti-staphylococcal agents when used against susceptible carrier strains. Other antibacterial agents were usually less effective unless they were used in higher concentration, more frequently or for longer periods. The suppressive effect of the antibiotics was more prolonged than that of the antibacterial

agents so that many carriers treated for a fortnight did not become recolonised for several weeks, For this reason intermittent treatment was successful. The antibacterial agents other than the antibiotics did not have a prolonged effect so that they required to be used continuously. There is thus need for an anti-staphylococcal agent of high potency with a residual effect abd suitable for topical application.

The use of the major therapeutic antibiotics for the suppression of Staph.aureus in carriers has disadvantages which are of greater importance inhospital than in the general community. Many of the carrier strains in hospital are resistant to these antibiotics and without a laboratory sensitivity test this cannot be foretold. The use of the correct antibiotic will require these tests.

Another danger is that drug-sensitisation may result from the repeated application of the antibiotics and this would be of disadvantage to the individual should he require the antibiotic therapeutically at a later date.

In addition strains of Staph. aureus resistant to the antibiotics will be encouraged to appear and spread by the mechanisms already discussed and dissemination of the antibiotic from the treated surface will contribute to the environmental antibiotic.

It is true that the same disadvantages apply to other anti-bacterial agents but they are of less practical importance. Strains of staphylococci resistant to these substances are not common at present, though they may increase. If so it is unlikely

that such strains will have clinical significance since they will not interfere with antibiotic therapy. Drug sensitisation may occur as with the antibiotics, though so far it is a rare occurrence. As parenteral administration of topical antibacterial agents is unlikely there is no great importance in this. Contamination of the environment will occur but will be of less importance importance than contamination with antibiotic.

The chief drawback to the use of antibacterial agents for the suppression of Staph. aureus is the difficulty in persuading healthy individuals to continue administration indefinitely. There can be little doubt however that if a large enough proportion of carriers in a community undertake this procedure diligently the dispersal of Staph.aureus and the number of infections can be reduced. It is easier to persuade patients with recurrent staphylococcal infection to apply antibacterial preparations to their carrier sites, and in the majority if the staphylococcus is suppressed, reinfection is prevented. Recolonisation will frequently occur when treatment stops and may be followed by reinfection.

To be fully effective in the hospital environment suppression of staphylococci in carriers must be coupled with other methods of preventing the dispersal of Staph.aureus and its access to new hosts. If all of these procedures are enthusiastically pursued the contamination of the environment can be reduced to a rate below that of removal from the environment. If under these conditions all cases of existing infection are removed there is a good chance of breaking the circle of spread of the organism and thereafter new infections will be reduced to a minimum.

SUMMARY

Nasal carriage of Staphylococcus aureus has been studied among staff and patients in hospital and among patients and medical students in the general community. Carriers were persistent or intermittent and were distinguished from those whose nares were temporarily contaminated with the organism by repeated examination and phage typing of the strains isolated.

Both carriers and those temporarily contaminated were more frequent in hospital than in the general community and the number of the latter was an indication of the degree of contamination of the environment with Staph. aureus. In hospitals examined in the Edinburgh area over 50% of the staff were nasal carriers and at any one time a further 15% were temporarily contaminated. In the general community some 30% of persons were carriers and less than 5% were temporarily contaminated.

Individual carriers were found to harbour the same staphylococcus for at least 4 years and it was calculated that the average length of time a young adult remains colonised is 14 years. The carrier rate was highest in infants; thereafter the nasal carriage rate fell during childhood and rose again during adolescence to the adult level which was almost the same at all age groups up to 50 years.

A limited study showed that sites other than the nose such as the perineum, groin and axilla are sometimes colonised, often in association with nasal carriage, less frequently as the sole site of colonisation.

The extent of contamination of reservoirs in the hospital and general community environments was examined and found to be proportional to the number of carriers and the presence of patients with staphylococcal lesions. Thus the dust in 100 cubic feet of air in private houses where no carriers of patients with staphylococcal lesions lived contained less than 0.1 Staph.aureus contaminated particle. The dust of most of the sites examined in hosptial contained at least 1o - 100 times this number and from maternity wards where 90% of the babies and 60% of the staff were carriers, 1000 times more Staph.aureus were isolated.

Nasal carriers were examined for dispersal of their staphylococci to the environment and it was found that large, though variable numbers were released by nearly all carriers. The main routes were via the hands by direct contact to new hosts or fomites, and as dust from the handkerchief and clothes. Breathing, speaking, coughing and sneezing liberated a few Staph.aureus from a few of the carriers and none from most.

Treatment of carriers with antibacterial preparations which were rubbed into the skin of the anterior nares, eliminated or reduced the number of Staph.aureus that could be recovered from the nose. As a result the number of staphylococci disseminated from a treated carrier was markedly reduced.

The strains of Staph.aureus isolated from infections among hospital staff, hospital patients and patients inthe general community were compared with strains isolated from carriers and the environments on the basis of their coagulase and penicillinase production and their susceptibility to antibiotics and bacteriophages.

The great majority of coagulase positive strains of staphylococci isolated from nasal carriers and patients with infections were susceptible to lysis by one or more of the bacteriophages used and so bacteriophage typing was a successful means of classifying those strains. A high proportion of coagulase-positive strains isolated from the dust and temporarily contaminatedpersons were not susceptible to the typing phages.

All coagulase positive strains able to grow in 0.1 unit per ml. of penicillin were found to produce penicillinase. It was therefore useful to group strains into penicillinase producers, which were regarded as penicillin-resistant (P:R) and non-penicillinase producers, which were regarded as penicillin sensitive(PS). This classification was useful since all the penicillin-sensitive strains were sensitive to streptomycin, chloramphenicol, the tetracyclines and erythromycin, and all strains resistant to one or more of these antibiotics was penicillinase producing and therefore penicillin resistant.

The antibiotic sensitivity pattern as shown by the susceptibility of a strain to a set of antibiotics was found to vary in some strains and therefore could not be used as a stable character or set of characters for classification purposes. Also the number of distinct patterns was limited only about 6 well-defined patterns being found among several thousand strains examined in the present work.

There was no definite association between phage type and antibiotic sensitivity pattern although very few antibioti-resistant strains were phage types belonging to Group II, and the majority of penicillin-resistant strains were of Group III types.

The presence of penicillin-resistant strains was relatively more frequent in carriers who had had antibiotic treatment than in those who had not. Penicillin-resistant strains were also rapidly acquired by persons in hospital or those who had frequent contact with hospital. Most infants were colonised within 8 days of birth, and many nurses in training were colonised within a few weeks of going into the wards. Penicillin-resistant strains were als o found in carriers in a factory environment where penicillin was being handled.

In both the factory and hospital environment free penicillin was recovered from the air-borne dust and from fomites, as well as from the hands and noses of personnel. Thus it has been concluded that environmental antibiotic is partly responsible for the emergence and spread of penicillin-resistant strains.

The number of phage 'types recovered in the hospitals examined was limited so that a few strains colonised the large majority of carriers and were found responsible for the majority of the hospital infedtion s. The phage types isolated have not

changed during the 7 years study covered by this investigation and there was no evidence of the introduction of fundamentally new types. There had however, been a chang in the proportion of some types in some hospitals where those of Group I have become predominant.

Over 90% of the strains isolated from carriers, patients and the environment in hospital were penicillin-resistant, and more than two-thirds were resistant to one or more of the other antibiotics. In contrast many phage types were isolated from carriers, patients and the environment in the general community. Only 25% were penicillin resistant and very few were resistant to the other antibiotics. The proportion of penicillin-resistant strains in the general community however was found to be considerably increased over the past 5 years and this was correlated with a change in the proportion of strains with phage types similar to those found in hospital. This may indicate a spread from the hospital to outside. Important in this respect are babies returning home heavily colonised with hospital strains and patients cross-infected with similar strains.

The strains of Staph. aureus isolated from patients with infections contracted in hospital were similar to those occurring in hospital carriers and the hospital environment. Relatively few of the patients examined were colonised in the nose with strains similar to those found in their lesions so that the majority of these hospital lesions must be cross-infections.

Ninety per cent of the staphylococcal infections examined in the general community occurred among carriers, and in 80% the lesion

and nasal strains were identical. Similarly staphylococcal infections occurred more frequently among nurses who were carriers than those who were not, and the nasal and lesion strains were identical in about 70% of cases. It seems most likely that these persons were the source of their own infections.

It was concluded that the most important source of staphylococcal infection in both hospital and general community was the carrier, in the majority of whom the nose was the primary site of colonisation with Staph.aureus. Auto-infection accounted for most infections in the general community and nurses in hospital, but for only a minority of infections in hospital infections in whom infection was the result of direct or indirect cross-infection from a carrier or case with a discharging lesion.

Antibacterial agents were thus used to suppress Staph.aureus in the noses of carriers and prevent his contamination and that of the environment. This was successful in preventing recurrence of staphylococcal infection in patients in general practice who were likely to be the source of their own infection. The treatment of hospital staff carriers had a similar effect in reducing the number of cases of cross-infection.

Nasal creams containing 1% of antibiotic or antibacterial agent such as chlorhexidene were applied to the nostrils at least 3 times a day for a fortnight and this served to suppress Staph. aureus in nearly 90% of carriers. The antibiotics had some residual effect so that continued suppression of the organism in the nose was possible by intermittent treatment. Other antibacterial agents such as chlorhexidene did not have this residual effect and required to be applied continuously.

The epidemiology of staphylococcal infections is complex and the causative organism may be intercepted at many points in its spread from source to susceptible host. Most methods of controlling these infections are designed to intercept Staph. aureus after it has dispersed from the host source. The present method is designed to prevent dissemination from the source so reducing contamination of both reservoirs and new hosts. It is suggested that the best results will be obtained by simultaneously using this method with others designed to prevent the spread of the organism in the environment.

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1: "The Determination of Bacterial Sensitivity to Antibiotics"
J.C.Gould & J.H.Bowie, Edinb.med.J.1952:59:178.

2: "Colouring Agents for use in Disc Diffusion Sensitivity Tests"
J.H.Bowie & J.C.Gould, J.Clin.Path.,1952:5:356.

3: "Carriage of Staphylococcus pyogenes in the Human Nose"
J.C.Gould & E.J.McKillop, J.Hygiene,1954:52:304.

4: "Staph.pyogenes: The Antibiotic Sensitivity of Strains isolated from the Human Nose".
J.C.Gould & E.J.McKillop, J.Hygiene,1954:52:486.

5: "The Prevention of Staph.pyogenes Cross-Infection by the Treatment of Carriers."
J.C.Gould & W.S.A.Allan, Lancet,1954:2:988.

6: "Erythromycin in the Treatment of Staph.pyogenes Infections. of the Newborn."
J.O.Forfar,C.L.Balf,A.F.Maccabe,H.A.Wright & J.C.Gould, Lancet,1955,1:584.

7: "The Origin of Antibiotic-Resistant Staph.pyogenes".
J.C.Gould. Nature,1955:176:176.

8: "The Effect of Local Antibiotics on the Nasal Carriage of Staph.pyogenes". J.C.Gould, J.Hygiene,1955:53:379.

9: "The Epidemiology of an Erythromycin-resistant Staphylococcus".
A.F.Maccabe, & J.C.Gould, Scot.med.J.,1956:1:223.

10: "The Origin of Antibiotic-resistant Staphylococci".
J.C.Gould,Nature, 1957:180:181.

11: "The Control of Carriers of Staph.aureus"
J.C.Gould, Symposium on Hospital Coccal Infections.
" 1957. Ass.Clin.Path. London., p.32.

12: Staphylococcal Infection in General Practice".
J.C.Gould, & J.D.Cruickshank, Lancet,1957:2:1132.

13: "Environmental Penicillin and Penicillin-resistant Staph.aureus".
J.C.Gould, Lancet,1958:1:489.

14: "The Epidemiology of Staphylococcal Infection."
J.C.Gould,1958, Abstracts, 7th. Int.Congr.Microbiol. Stockholm, p.351.

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REFERENCES

ABRAHAM,E.P.,CHAIN,E.,FLETCHER,C.M.FLOREY,H.W.,GARDNER,A.D., HEATLEY,N.G.,& JENNINGS,M.A. 1941, Lancet,1:652.

ABD-EL-MALEK,Y.,& GIBSON,T. 1948, J.Dairy Res.,15:249 .

ALLISON,V.D., & HOBBS,B.C. 1947, Brit.med.J., 2:1.

ANDREWES,F.W., & GORDON M.H. 1905, Ann. Rep.Local Govt.Board. Rep.Med.Off. Append.No.7. 35:543.

ASHESHOV,E.H., & RIPPON, J.E. 1959, J.Gen.Micrbiol. 20:634.

BARBER,M. 1947, J.Path.Bact., 59: 373.

------& ROZWADOWSKA-DOWZENKO,M., 1948, Lancet,2: 641.

------ 1949, J.Gen. Microbiol., 3: 274.

------, HAYHOE,F.G.J.,& WHITEHEAD,J.E.M. 1949, Lancet,2:1120.

BEAVEN,D.W. & BURRY,A.F. 1956, Lancet,2:211.

BELLAMY,D.W., & KLIMEK,J.W. 1948,J.Bact.,55:153.

BERRIDGE,N.J. 1956, J.Dairy Res.,23:336 .

BIGGER,J.W. 1944, Irish J.med. sci. Ser. 6:553.

BILLROTH, T. 1874, Untersuchungen über die Vegetationsformen von Coccobacteria Septica. Reimer. Berlin.

BLAIR,J,E., CARR,M. &BUCHMAN J., 1946, J.immunol.,52:281.

BLOOMFIELD,A.L., 1921, Bull.Johns Hopk. Hosp. 32:290.

BLOWERS,R., POTTER,J., & WALLACE,K.R. 1957, Lancet,2:629.

BONDI,A., & DIETZ,C.C., 1945, Proc.soc.exp.Biol.(NY). 60:55.

---- PfAFF,F.,FREE,E., & SWERLICK,R. 1954, Amer.J.Pub.Hlth.44:789.

BOWIE,J.H. 1953, Trans.Edin.Obstet.Soc., 106:1.

--- & GOULD,J.C. 1952, J.Clin.Path. 5:356.

BOYCOTT,J.A. 1956, Lancet, 2: 678.

British Medical Journal,1955, 1:591.

BRODIE,J., & KERR,M.R., & SOMERVILLE,T., 1956, Lancet,1:19.

--- SOMERVILLE,T., & WILSON,S.G., 1956,Brit.med.J. 1: 667.

BURNETT,W.E.,CASWELL,H.T.,SCHRECK,K.M.,CARRINGTON,E.R.,LEARNER,N., STEEL,H.H.,TYSON,R.R., & WRIGHT,W.C. 1958,J.Amer.med.Assn.166:1178.

BURNET,F.M., & LUSH,D. 1935, J.Path.Bact.,40:445.

BURROWS,A.,RUSSEL,B., & MAY,H.B. 1945,Brit.J.Dermatol.,57: 97.

CADNESS-GRAVES,B. WILLIAMS,R., HARPER,G.J. & MILES,A.A., 1943, Lancet, 1:736.

CAIRNS,H.J.F., & SUMMERS,G.A.C. 1950, Lancet,1:446.

CHAIN,E. & DUTHIE,E.S., 1945, Lancet,1:652.

CHALLINOR,S.W. 1943, J.Hyg.(Camb.), 43:16.

CHANDLER,C.A.,DAVIDSON,V.Z.,LONG.P.H., & MONNIER,J. 1951, Bull.Johns.Hopk.Hosp. 89:81.

CHAPMAN,G.H. 1945, A J.Bact. 50: 119.

CHRISTIE,R., & KEOGH, E.V., 1940, J.Path.Bact., 51: 189.

CLARKE,S.K.R. 1957, J.Path.Bact., 73:253.

COWAN, S.T. 1955, Int.Bull. bact. nomencl.taxon. 6:99.

COLBECK,J.C. & SUTHERLAND,W.H. 1958, Canad.J.Surg. 1:8.

COLEBROOK, L. 1950, " A new approach to the treatment of burns and scalds" Fine Technical Publications, London.

COOK,J., PARRISH,J.A., & SHOOTER,R.A. 1958, Brit.med.J. 1:74.

COWAN,ST. 1938, J.Path.Bact. 46: 31 .

---- 1939, J.Path.Bact. 48:169.

CRAIGIE,J. & YEN,C.H. 1938, Amer. Pub.Hlth.J. 29:448.

CRUICKSHANK, J.D. Personal communication.

CRUICKSHANK,R.C. 1937, J.Path.Bact., 45: 295.

CUMMINS,S.L., & CUMMINS,C.C. 1913, J.Roy.Army.med.Corps,20:499.

CUNLIFFE,A.C. 1949, Lancet,2:411.

DARANYI,J.von, 1925, Arch.Hyg. (Berl.) 96:182.

DARANYI,J. 1955, Arch.Hyg.(Berl) 139:3.

DEMEREC,M. 1945 a. Proc.Nat.Acad.sci.(Wash.) 31:16.

--- 1945 b. Ann.Mo.Bot.Gdn., 32:131.

--- 1948, J.Bact., 56:63.

DEVENISH,E.A., & MILES,A.A. 1939, Lancet,1:1088.

De Waal,H.L. 1940, J.Hyg.(Camb.) 40:172.

DOWLING,H.F.,LEPPER,M.H., & JACKSON,G.G. 1953, Amer.J.Pub.Hlth.,43:860.

DuBUY, H.G., CRISP,L.R. (1944). Publ. Hlth. Rep Wash. 59: 829

DUGUID,J.P. 1946, Edinb.med.J., 53:401.

--- & WALLACE,A.T. 1948, Lancet, 2:845.

DUNCAN,J.T. & WALKER,J. 1942, J.Hyg.(Lond.) 42:474.

EDMUNDS,PN.,ELIAS-JONES,T.F.,FORFAR,J.O., & BALF,C.L. 1955, Brit.med.J.,1:990.

ELEK,S.D. 1959, "Staph.pyogenes and its relationship to human Disease" . E.S.Livingstone, Edinburgh.

---- & CONEN,P.E. 1957, Brit.J.exp.Path., 38:573.

ELWOOD,J.S. 1951, J.Hyg.(Lond.), 49:263.

ERIKSEN,K.R. 1949, Acta.Path.Microbiol.Scand., 26:269.

FAIRBROTHER,R.W. 1956, Lancet, 1:716.

----- & SOUTHALL, J.E. 1950, Month.Bull.Minist.Hlth. Lond. 9: 170.

FINLAND,M., FRANK,P.F., & WILCOX,C. 1950, Amer.J.Clin.Path.20:325.

--- & HAIGHT,T.H. 1953, Arch.Int.med., 91:143.

FISK,A. 1940, Brit.J.Exp.Path. 21:311.

FISK, R.T. 1942a, J.Infect.Dis., 71:153.

--- 1942b, J.Infect.Dis., 71:101.

FLEMING,A. 1929, Brit.J.Exp.Path., 10:226.

FLOREY,H.W.,CHAIN,E.,HEATLEY,N.G.,JENNINGS,M.A.,SANDERS,A.G., ABRAHAM,E.P. & FLOREY,H.E., 1949. "Antibiotics" Oxford Univ. Press,Lond.

FORFAR,F.O. & MACCABE,A.F., 1958,Brit,med.J. 1:76.

FOSTER,J.W. & WOODRUFF,H.B. 1943,J.Bact., 46: 187.

---- --- 1944, J.Bact., 47:43.

FUJITA,A, & YOSHIOKA,S. 1938, Jap.J.Med.Sci. 1:46.

FUSILLO,M.H., ROERIG,R.N.,7 ERNST,K.F., 1954,Antibiot.Chemotherap.4:1202.

GALE,E.F. 1947a " The chemical Activities of Bacteria" Univ.Tutorial Press.

-------- 1947 b. J.Gen.microbiol. 1:327.

------- & RODWELL,E.W. 1949, J.Gen,Microbiol. 3:127.

GALLARDO,E. 1945, War Med., Chicago. 7:100.

GERHEIM,E.B., & FERGUSON,J.H. 1949, Proc.soc.exp.Biol.(N.Y.),66:1525.

GILLESPIE,E.H., DEVENISH,E.A. & COWAN,S.T., 1939, Lancet,2:870.

GILLESPIE,W.A.,SIMPSON,K.,7 TOZER,R.C. 1958, Lancet,2:1075.

GILSON,B.ST.C., & PARKER,R.F., 1948, J.Bact., 55:801.

GOLDBERG,H.S., & MASTERTON,B.J. 1956, Antibiotics Annual,1956-57, 4:667.

GOHAR, M.A., (1951) J.Roy. Egypt Med.Assn. 34:105.

GORDON,M.H., 1903, Rep.Loc.Govt.Brd. Rep.med.Off. 33:388.

GOSLINGS,W.R.D, BUHLI,K. 1958, Arch.Int.med. 102:691 .

GOTS,J.S., 1945, Science,102,309.

GOTZ,H. & RUNG,PA., (1951Klin. Wschr. 29:55 .

GOULD,J.C., & BOWIE,J.J. 1952, Edinb.med.J. 59:178.

GOULD,J.C.,BOWIE,J.H., & CAMERON,J.D.S. 1953, Lancet, 1:361

GOULD,J.C., & CRUICKSHANK,J.D. (To be published).

GRAESSLE, O.E., & FROST,B.M., 1946, Proc.soc.exp.Biol. 63:171.

GREENDYKE,R.M.,CONSTANTINE,HP.,McRUDER,G.B.,DEAN,D.C.,GARDNER,J.N., & MORGAN,H.R., 1958, Amer. J.Clin.Path. 30:318.

HALLMAN,F.A., 1937, Proc.soc.exp.Biol., 36: 789.

HARE,R.,& THOMAS,C.G.A., 1956, Brit.med.J.,2:840.

HARE,R., & RIDLEY,M.,1958, Brit.med.J.,1:69.

HARRISON,M.H.M., 1948, Lancet, 2:727.

HART,D., 1937, Arch.surg. 34: 824.

HARTLEY,L.,& BOWIE,J.H., 1946, Brit.med.j.,1:639.

HENNESSEY,R.S.F., & MILES,R.A., 1958, Brit.med.J. 2:893.

HOBBS,B.C., 1944, Month.Bull.Minist. Hlth. 3:11.

---- 1948, J.Hyg.Lond., 46:222.

--- CARRUTHERS,H.L., & GOUGH,S., 1947,Lancet,2:572.

HOWE,C.W., 1954, New Eng.J.med., 251:411.

--- 1956, ibid 255:787.

HURST,V., 1957a J.Hyg.(Camb)., 55:299.

--- 1957b ibid 55:313.

HUTCHISON,J.G.P., GREEN,C.A., & GRIMSON,T,A.,1957,J.Clin.Path.10:92.

INGRAM,G.I.C., 1951, J.Gen.Microbiol. 5:22.

ISBISTER,C., DURIE,E.B.,ROUNTREE,P.M., & FREEMAN,B.M .,1954, Med.J.Austr. 2: 897.

Jour.Amer.med.Assoc. 1957, Queries and Minor Notes, 163:904.

JEFFREY,J. & SKLAROFF,S.A. 1958, Lancet, 1 : 365.

JOHANOVSKY,J., 1958, Abstracts 7th.Int.Congress Microbiol. Stockholm. 355.

JULIANELLE,L.A. 7 WIEGHARD,C.W., 1934, Proc.soc.exp.Biol.(N.Y.) 31:947.

KIRBY,W.M.M. 1945, J.Clin.Invest., 24:165.

--- & AHERN,J.J., 1953, Antibiot. Chemotherap., 3:831.

KLIMEK,J.W., CAVALLITO,C.J., & BAILLEY,J.H., 1948, J.Bact.,55:139.

KNEELAND,Y(Jr.), 1930, J.Exp.med., 51,617.

KNIGHT,V., HOLZER,A.R., 1954,J.Clin.Invest. 33:1190.

--- WhITE,A.,FOSTER,F. & WENDZELL,T. 1956, Ann.N.Y.Acad., sci., 65: 206.

KNOTT,F.A., & BLAIKELY,J.B. 1944, J.Obstet.Gynaec.,51:386.

KOLMER,J.A. 1947, South. med.J. 40: 72.

LAURELL,G. & WALLMARK,G., 1953a Acta.Path.Microbiol.Scand.32:424.

LAURELL,G. & WALLMARK,G., 1953b Acta.Path.Microbiol.Scand.32:432.

LEPPER,M.H.,DOWLING,H.I.JACKSON,G.G. & HIRSCH,M. 1953,Arch.Int.med.92:40

--- JACKSON,G.G. & DOWLING,H.F.,1955. J.LAB.CLIN.MED.45:935.

LESVEQUE,J.,PECKER,Mme.E., 1952, Nourisson, 40:189.

LOEB,L. 1903, J.med.Res., 10:407.

LOH,Wei-Ping, & ABIOG,I. 1957, New Eng.J.Med.256:179.

LOWDEN, T.G., 1954, Lancet,2:1175.

LOMINSKI,I. & GROSSFELD,E., 1944, Brit.med.J., 2:854.

----- ------ 1948, Brit.med.J., 1:343

------ & ROBERTS,G.B.S.,1946, J.Path,Bact., 58:187.

LUDLAM,G.B., 1949, Month.Bull. Min.of Hlth., 8: 15.

--- 1953, J.Hyg,Lond, 51:64.

LURIA,S.E., 1946, Proc.soc.exp.Biol.(N.Y.), 61:46.

MACCABE,A.F., 1959, Scot.med.J. 4:75.

MacFARLAN,A.M., 1938, Brit.med.J. 2:932.

MACKIE,T.J.,McCARTNEY,J.E.,1958, "Handbook of Practical Bacteriology" 9th.Edition, E.&S. Livingston, Edinburgh.

MAITLAND,H.B., & MARTYN,G. 1948, J.Path.Bact. 60:553

MARKHAM,N.P., 1958, New Zeal.Med.J. 57:317.

--- 1959 Personal communication.

MARTIN, T.D.M. & WHITEHEAD,J.E.M., 1949, Brit.med.J. 1:173.

MARTYN,G. 1949, Brit.med.J. 1:710.

MAY,J.R., & OSWALD,N,C., 1956,Lancet,6:814.

MEDICAL RESEARCH COUNCIL, 1951. "The control of CrossInfections in Hospitals" Memo No 11. H.M.S.O.

MILES,A.A., 1941, Lancet,2:507.

MILES,A.A., WILLIAMS,R.E.O., 7 CLAYTON-COOPER,B., 1944, J.Bath.Bact. 56:513.

MITCHELL,A.A.B.,TIMBURY,M.C.,PETTIGREW,A.G., & HUTCHISON,J.G.P., 1959,Lancet,2:503.

MONNIER,J.J. & SCHOENBACH,E.B., 1951, Antibiot.Chemotherap. 1:472.

MORLEY,D.C., 1945, J.Path.Bact, 57, 379.

MOSS, B, SQUIRE,JR.,TOPLEY,E, & JOHNSTONE,C.M., 1948, Lancet,1:320.

MUCH,H. 1908, Biochem.Z. 14:143.

MURDOCH, J.McC.LECKIE,W.J.M., DOWNIE,J.,SWAIN,R.H.A. & GOULD,J.C., 1959,Brit.med.J. (in press).

NEEDHAM,G.M., & NICHOLS D.R., 1953,J.Lab.Clin.Path.med. 41:150 .

NELSON,M.G., 1952, Ulster med.J., 21:36.

NOLAN,W.A., & FLEISCHER,H.B., 1958,New Eng. J. Med. 258:490.

North,E.A. & CHRISTIE,R. 1945, Med.J.Austr. 33 44.

----- ------ 1946, ibid 33:176.

------ ----- & RANK,B.K. 1946, Med. J. Austr.2:43.

NUFFIELD HOSPITAL REPORT "Studies in the function and design of hospitals")Nuffield Prov.Hosp.Trust. O.U.P. 1955.

CEDING,P., 1952, Acta.Path.Microbiol.Scand., Suppl. 93:356.

---- 1954, ibid. Suppl. 37:116.

OGSTON,A. 1880. Arch. Clin. Surg. 25:588.

---- 1881. Brit.med.J. 1: 369.

--- 1882. J.Anat. Lond. 16:526.

----- 1882 ibid 17:24.

OSWALD,N.C., SHOOTER,R.A., & CURWEN,M.P. 1958, Brit.med.J. 2:1305.

PARKER,R.F., 1946, Proc.soc.exp.Biol.(N.Y.) 63: 443

PARKER,M.T.,TOMLINSON,A.J., & WILLIAMS,R.E.O., 1955, J.Hyg.(Lond.) 53:458.

PLOUGH,H.H., 1945, Amer.J.Clin.Path. 15:446.

POPE, Quoted by Heatley,N.G. (1944) J.Biochem,38:61.

RAKE,G., McKEE,C.M., HAMRE,DM. & HOUCK,C.L., 1944, J.immunol,48:271.

RAMMELKAMP,C.H., & MAXON,T., 1942, Proc.soc.exp.biol. 51:386.

RAVENHOLT,R.T., & LAVESQUE,G.D. 1956, Amer.J.Pub.Hlth. 46:12/87.

ROSENBACH,F.J., 1884 Microorganismen bei den Wundinfection-krankeiten des Menschen. Weisbaden.

ROUNTREE,P.M., 1959, J.Gen. Microbiol. 20: 620

--- 1956, Lancet,1:719.

---- 1953, Atti del 6th.Congr. Int di Microbiol.Roma. v.3,p.489.

--- BARBOUR,R.G.H., 1951, J.Path.Bact. 63:313.

---- ------ THOMSON,E.F., 1951,Lancet,1:435.

---- THOMSON,E.F., 1949, Lancet,2:501.

---- FREEMAN,B,M., & BARBOUR,R.G.H., 1954,Med.J.Austr.2:457.

----- ------- JOHNSTON,K.A., 1956,J.Path.Bact,72:319.

RUBBO,S.D. & BENJAMIN,M. 1951, Brit.Med.J.1:983.

SAINT- MARTIN,M., 1953, Canad. J.Pub.Hlth.,44:324.

SHOOTER, R.A.,GRIFFITHS,J.D.,COOK,J.D., & WILLIAMS,R.E.O.,1957, Brit.Med.j. 1:433.

SMITH,C.A., 1935, Lancet,1: 1204.

SONNENSCHEIN,C. L927, Zbl. Bakt. I.Abstr.Orig. 104:365.

SPINK,W.W., & VIVINO,J.J. 1944, J.Clin. Invest. 23:267.

--- FERRIS,V., 1947, J.Clin.Invest. 26:379.

SPINK,W.W., 1956, Annua N.Y.Acad.Sci. 65. 175.

STORRS ,F.C. & HIETT- BROWN,W. L(*' 1954, J.Dairy Res.,21:337.

SUMMERS,G.A.C., 1952, Lancet,1:135.

SWINGLE,E.L., 1935, J.Bact. 29: 467.

SZYBALSKI,W., 1953, Antibiotic & Chemotherap. 3: 195.

THOMAS,E.G.A., LIDDELL,J. & CARMICHAEL,D.S. 1958, Brit. med.J.2 :1336.

THOMSON, B.A., 1950, J.Clin.Path, 3:118.

THOMSON,.M.E.M., & GILLESPIE, W.A., L(*' 1958,J.Path.Bact.75:35.

THOMSON ST.CLAIR.,& HEWLETT,R.T., 1895. Trans Med Chir,Soc Lond. 78:239.

THOMSON ,B.A., SCHWABACHER,H., 1951, J.Clin.Path.4:350.

TIMBURY,M.C.,WILSON,T,S, HUTCHISON,J.G.P. & GOVAN A.D.T., 1958., Lancet,2:1081.

TORREY,J.C. & REESE.M.K.,L(*£ 1945, Amer.J.Dis.Child. 69:208.

TURPIN,R.,PILLET,J., & PILLET Mme. J., 1953 , Arch.France.Paediat. 10:988.

VERNEUIL, 1880, Arch Gen. Med. 146:641.

VERNEY,R, Personal communication.

VOGELSANG,T.M. AND BOE,J. , 1952, Acta,Path,microbiol, Scand. Suppl. 93 :349.

VogelsgangT.M., & Boe J., 1953 Acta. Path. Microbiol, Scand.33: 294.

VOUREKA, A. AND HUGHES,W.H.,1944 Brit.med.J. 1:395.

WAHL,P. & LAPEYRE?MESIGNAC P., 1950, Ann.Inst.Past., 78:353.

WALKER,W.C., DOUGLAS A.C., LECKIE, W.J.M.,PINES,A. & GRANT W.B., 1958, Lancet,1: 449.

WALLMARK,G., 1954, Acta.Path.microbiol scand,34:57.

Wear, L.E., 1958. Lancet,1:477.

WIALLIAMS,J.R.B.,AND TALBOT,E.C.S, AND MAUGHAM,E., 1959, Brit.med.J. 1:1374.

WILLIAMS,R.E.O., 1946, J.Path.Bact., 58: 259.

------ & RIPPON, J.E. 1952, J.Hyg.Lond. ,50:320.

------ ------ & DOWSETT,L.M.,1953,Lancet,1 510.

WILLIAMS,S. & TIMMINS C, 1938, Med.J.Austr., 25:687.

WILSON,G.S., & ATKINSON, J.D., 1945, Lancet,1:647.

WISE ,R. , 1958, J.Amer.Med.Assn. 166:1178.

------ GRANNY ,C.C., & SPINK,W, 1954, J.Lab.Clin.med.44:956.

WRENSHALL,C.L.,1956, Antibiotics annual, 4:809.

WYSHAM, O.N. & KIRBY, W., 1957, J.amer. med,assn.,164,1733.